

**CERTAIN ASPECTS OF REPRODUCTIVE BIOLOGY OF
THE GHOST CRAB, *OCYPODE PLATYTARSIS* WITH
SPECIAL REFERENCE TO THE MALE**

T H E S I S

**SUBMITTED TO THE FACULTY OF SCIENCE, UNIVERSITY OF CALICUT
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ZOOLOGY**

by

M. SUKUMARAN, *M Sc.*,

September, 1985

Department of Zoology, University of Calicut

Kerala, 673635, India

DEPARTMENT OF ZOOLOGY
UNIVERSITY OF CALICUT



71 (Cholari)
CALICUT UNIVERSITY P. O.
Kerala 673 038
Date... 30 - 9 - '85

C_e_r_t_i_f_i_c_a_t_e

This is to certify that this thesis is an authentic record of the work carried out by Sri. M. Sukumaran, M.Sc. from January, 1981 to December, 1984 under my supervision and guidance in partial fulfilment of the requirements of the degree of Doctor of Philosophy under the Faculty of Science of the University of Calicut. No part of this thesis has been presented before for any other degree.

Prof. Dr. K. G. Adiyodi
Department of Zoology
Calicut University
Kerala-673635, India

A c k n o w l e d g e m e n t s

I express my profound sense of gratitude and obligation to Prof. Dr. K.G. Adiyodi, for suggesting the problem, his invaluable guidance, eminent supervision and unceasing encouragements throughout the course of this investigation. I am highly indebted to Prof. Dr. R.G. Adiyodi for much constructive criticism, valuable advice and all other help. My sincere thanks are due to Prof. Dr. K.J. Joseph, Head of the Department of Zoology, for providing laboratory facilities and other requisites.

I am thankful to Prof. Dr. Wolfe Engels, Institute for Zoology, University of Tübingen, West Germany, for facilities extended to take some of the photomicrographs. The technical assistance by the staff of Art and Photography Unit, Calicut University and Beena Commercial Institute, Calicut University is also acknowledged.

I am extremely grateful to Dr. S. Sethuramalingam, Porto Novo for identifying the species of crabs used in this study.

I acknowledge with thanks the financial assistance received from the University Grants Commission (Grant No.F.23-1083/79-SRII). I take this opportunity to express my deep sense of gratitude to my colleagues and friends for their co-operation and help.

M. Sukumaran

C o n t e n t s

Page

I. Reproductive biology of male Crustacea: A review

1.1	Introduction	1
1.2	Composition of seminal plasma	2
1.3	Sperm transfer strategy	6
1.4	Semenogenesis	14
1.5	Accessory sex glands and their functions	18
1.6	Spermatogenesis and sperm function	23
1.7	Fate of semen in the female system	30
1.8	Reproductive cyclicity and role of exogenous factors	32
1.9	Endogenous factors influencing reproduction	35
1.9.1	Role of neurohormones	36
1.9.2	Androgenic glands and androgenic hormones	40
1.9.3	Reproductive metabolism	45
1.10	Concluding remarks	46

II. Biology of the ghost crab, Ocypode platytarsis Milne Edwards

2.1	Introduction	49
2.2	Materials and methods	50
2.2.1	Method of collection	50
2.2.2	Mode of transport	54
2.2.3	Laboratory culture	55
2.3	Observations	55
2.3.1	Animals	55
2.3.2	Habits	57
2.3.3	Food and feeding pattern	60
2.3.4	Habitat	62
2.3.5	Breeding and life cycle	65

	Page
2.4 Discussion	66
2.5 Summary	74
III. Certain aspects of semenogenesis in <u>Ocypode platytarsis</u> Milne Edwards	
3.1 Introduction	76
3.2 Materials and methods	76
3.2.1 Animals	76
3.2.2 Tissue analysis	77
3.2.3 Histochemical analysis	78
3.3 Observations	80
3.3.1 Gross morphology of the male genital system in <u>O. platytarsis</u> .	80
3.3.2 Spermatozoa in <u>O. platytarsis</u>	81
3.3.3 Histophysiology and histochemistry of sperm duct in <u>O. platytarsis</u> : aspects of spermatophore formation	81
3.3.4 Coral-shaped accessory sex gland	103
3.3.5 Mode of semen production in <u>O. platytarsis</u>	106
3.3.6 Semen in spermatheca	110
3.4 Discussion	117
3.5 Summary	138
IV. Chemical characteristics of the reproductive system and hepatopancreas of male <u>Ocypode platytarsis</u> Milne Edwards	
4.1 Introduction	143
4.2 Materials and methods	144
4.2.1 Qualitative analysis	144
4.2.2 Quantitative analysis	145
4.3 Observations	146
4.3.1 Male reproductive system	146
4.3.2 Hepatopancreas	150

	Page
4.3.3 Primary semen	151
4.4 Discussion	165
4.5 Summary	185
V. Seasonal variation in male reproductive system of <u>Ocypode platytarsis</u> Milne Edwards	
5.1 Introduction	189
5.2 Materials and methods	190
5.3 Results	191
5.3.1 Seasonal variations in spermatogenesis	191
5.3.2 Seasonal variations in androgenic glands	195
5.3.3 Seasonal fluctuations in vas deferens and CSG of <u>O. platytarsis</u>	197
5.4 Discussion	212
5.5 Summary	228
VI. Endocrine control of reproduction in male <u>Ocypode platytarsis</u> Milne Edwards	
6.1 Introduction	232
6.2 Materials and methods	234
6.2.1 Eyestalk ablation experiments	234
6.2.2 Implantation experiemnts	236
6.3 Results	237
6.3.1 Effect of eyestalk ablation on CSG of <u>O. platytarsis</u> during September/October	238
6.3.2 Effect of eyestalk ablation on the vas deferens of <u>O. platytarsis</u> during September/October	239
6.3.3 Effect of eyestalk ablation on the CSG of <u>O. platytarsis</u> during March/April	241
6.3.4 Effect of eyestalk extirpation on vas deferens of <u>O. platytarsis</u> during March/April	242

	Page
6.3.5 Effect of the eyestalk ablation on androgenic glands of <u>O. platytarsis</u>	244
6.3.6 Role of androgenic gland in maintenance of CSG of <u>O. platytarsis</u>	245
6.4 Discussion	282
6.5 Summary	295
References	300

Chapter I

Reproductive Biology of male Crustacea : A review

M Sukumaran “Certain aspects of reproductive biology of the ghost crab, *Ocypode Platyt Aris* with special reference to the male” Thesis. Department of Zoology, University of Calicut, 1985

1.1. INTRODUCTION

The arthropods make about 80% of all species of animals living today and are the most successful of all invertebrates. Crustaceans constitute only a comparatively small group of the Arthropoda; some species such as the prawns, crabs and lobsters are greatly valued as delicacies of the table. There has been recently a growing interest in various aspects of the biology of crustacean reproduction. This was triggered, on the one hand, by the rapid advance made by insect reproductive biology and endocrinology, and on the other by the realization that a knowledge of reproductive biology is essential to aquaculture and scientific management of useful crustacean species. Biology of reproduction in female crustaceans has been studied in some detail and reviewed frequently (see K.G. Adiyodi and R.G. Adiyodi, 1970, 1975, 1982; R.G. Adiyodi, 1978, 1985; R.G. Adiyodi and Subramoniam, 1983), but much less is known on males, particularly on mechanisms controlling their reproduction. Some data are available on morphology and fine structure of the male reproductive system and on spermatogenesis and structural details of spermatozoa but surprisingly very little is known on the biochemistry and physiology of semen, formation of spermatophores, and the cyclicity in physiology of the male reproductive

system in relation to different stages in the moulting cycle and seasons. We are also in the dark regarding the degree of dependence of male Crustacea on eyestalk principles for their reproductive activity. This chapter gives a state-of-the-art-review of the biology of reproduction in male Crustacea and highlights some gaps in our knowledge which prompted us to undertake this work.

1.2. COMPOSITION OF SEMINAL PLASMA

Though the degree of importance of seminal plasma in successful gamete union varies in invertebrates according to the type and time of fertilization, it is clear that in many groups the natural copulatory process resulting in transfer of male gamete cannot take place smoothly and efficiently without seminal plasma, the quantity of which varies in different species. In insects, the seminal plasma is known to contain in some species chemicals that enhance fecundity of females and also inhibit their receptivity (Gillott and Friedel, 1977 for review). Barnes (1962a) studied the nature of the seminal plasma of the barnacle, Balanus balanus. The cirripede semen (in seminal vesicle) is a sticky, viscous fluid (pH: 7.3-7.4) with a density of 1.041-1.044. It coagulates in contact with water. TCA-precipitable

proteins form about 35.1% of the total solids excluding inorganic ions in the seminal plasma. Ascorbic acid content is 21 $\mu\text{m}/\text{ml}$ and glycogen and glucose together 1.05 mg/ml. Potassium and calcium are present in excess of their quantities in sea water. Total free amino acid content of the semen of B. balanus is much less than that of body extracts. Glycine, sarcosine, taurine and proline are the major free amino acids in seminal plasma of B. balanus (Barnes and Blackstock, 1974). Phosphorus of all kinds is low. The seminal plasma of B. balanus reacts positively for ATP-ase and 5'-nucleotidase but no phosphomonoesterases have been detected. The presence of glycogen, glucose and small quantities of lactic acid in semen suggests that glycolysis may take place in natural semen of B. balanus (Barnes and Finlayson, 1962; Barnes and Blackstock, 1974), though Barnes and colleagues have not described all the key enzymes and intermediate compounds in the pathway.

In the brine shrimp Artemia salina, the seminal fluid contains neutral mucopolysaccharides or mucoproteins (Wolfe, 1971). Ultrastructural studies reveal that the seminal fluid of Libinia emarginata is composed of two components; (1) an electron-dense substance and (2) large granules (in limited numbers) with a central core of varying density and a periphery containing particulate

matter (Hinsch and Walker, 1974), Mouchet (1931) had reported earlier that the vas deferens of both *Anomura* and *Brachyura* secreted a chitin-free fluid that could be coagulated by various means. Greenwood (1972) observed an inter-spermatophoric mucous secretion in Pagurus sp. Seminal fluid in Portunus is comprised of at least three layers of material (Ryan, 1967).

Studies in our laboratory on the prawn Penaeus indicus have shown that the seminal plasma stored in major compartment of the seminal vesicle is a colourless and translucent fluid. It is basically proteinaceous and has a high content of sulphated acid mucopolysaccharides (Muthuraman and K.G. Adiyodi, 1981; Muthuraman, 1985). Tyrosine-containing proteins form the major part of the seminal plasma of Macrobrachium idella; periodate-reactive and periodate-unreactive mucosubstances, lipids, and moderate quantities of glycogen are also present (Sreekumar, 1985). Spermi-ductal secretion in the freshwater crab, Paratelphusa hydrodromous is slightly acidic and proteinaceous. It contains proteoses and free amino acids. Glycogen is present in small quantities; dextrans, reducing sugars, sulphated acid mucopolysaccharides and lipids are either absent or are present only in low levels (Sabeeda et al., 1974; Mathad and K.G. Adiyodi, 1979). Spermatophores are

suspended in a fluid which is rich in basic protein and contains neutral lipid droplets, glycogen-rich globular units and highly basophilic proteinaceous droplets (Mathad, 1983). In another freshwater crab, Barytelphusa cunicularis, the seminal plasma is a mildly acidophilic secretion composed of droplets of neutral lipid, alcianophilic substances and periodate-reactive non-alcianophilic components (Mathad and K.G. Adiyodi, 1980; Mathad, 1983).

In summary, the seminal plasma of crustaceans appears to be largely a proteinaceous product with varying amounts of mucopolysaccharides, glycogen, lipid and possibly some free amino acids. A comparison of the composition of seminal plasma of crustaceans with that of other invertebrates and vertebrates could be of great interest, but unfortunately detailed information on composition of seminal plasma is available for few crustacean species. In the honeybee Apis mellifera, the seminal plasma which is a product of the seminal vesicle, mucous gland and penis bulb contains at least three free sugars - fructose, glucose and trehalose. In Balanus semen, the main free sugar is glucose whereas in mammals is fructose which is conspicuous by its absence in Balanus semen. In addition to coagulable proteins, the seminal plasma of Apis mellifera contains

an impressive range of free amino acids (see Taber, 1977 for details). Glutamic acid, alanine, glycine, serine and aspartic acid are the chief amino acids present in the seminal plasma of cock, bull, ram, goat and man, whereas in Balanus seminal plasma, the chief amino acids are glycine, sarcosine, taurine and proline. Proteolysis of ejaculated semen is rapid in man but slow in B. balanus. More studies are needed on chemical composition of the seminal plasma of crustaceans and on physiological role of various chemical constituents, for the spermatophore remain suspended in this medium within the male genital system and are delivered mixed with seminal plasma to the female in species fertilizing their eggs internally.

1.3. SPERM TRANSFER STRATEGY

In crustacean semen, the spermatozoa are usually seen enclosed within spermatophores. The spermatophores are protective devices evolved by some groups (e.g. Annelida, Arthropoda) for delivery of spermatozoa from males to females (K.G. Adiyodi and R.G. Adiyodi, 1975, 1983; Mann, 1984). In Crustacea, the spermatophores may be stalked (pedunculate) or non-stalked. Stalked spermatophores are common among the Anomura, Greenwood (1972) has described different stages in formation of

the stalked spermatophores of Pagurus novae-zealandiae. First of all, an acidophilic hyaline material produced by the second region of the vas deferens is deposited as a sperm sheath around the sperm mass. In the third part of the sperm duct, the sperm mass gets divided into separate, individual capsules containing a definite number of spermatozoa due to peristaltic contractions of the musculature, resistance of formed spermatophores, folding of the sheath and buckling of the weaker areas. Afterwards, a sticky and granular pedestal-forming material gets attached to the foot and the neighbouring feet are united into a pedestal. Lastly, the final form of spermatophore is carved and due to vigorous muscular contractions the pedestal is segmented off with a number of spermatophores attached to it.

The pedunculate spermatophores of Emerita asiatica are dimorphic and are attached by the pedunculate end in a row on strands of a membrane, which is embedded in jelly-like galatinous matrix (Subramoniam, 1981). In Albunea symnisia (Anomura), the spermatophore is not pedunculate, but in the form of a highly convoluted tube set on a firm membrane forming a continuous cord-like mass. The spermatophoric ribbon in A. symnisia is surrounded by a putty-like gelatinous matrix as in Emerita asiatica (Subramoniam, 1981).

In many groups of Crustacea including the Brachyura, non-stalked spermatophores are observed. They vary widely in size and shape. The shape varies from cylindrical (Derocheilocaris typicus: Brown and Metz, 1967) and arch-like (Aniculus sp.: Mouchet, 1931) to oval (Scylla serrata: Uma and Subramoniam, 1979; Paratelphusa hydrodromous: Mathad and K.G. Adiyodi, 1979). In Penaeus indicus, the spermatophore is tripartite. The spermatozoa are lodged in the central C-shaped unit to which are attached two wing-like processes. Proteins (7.263%), free amino acids (0.380%), and ammonia (39 µg/100 mg wet weight) have been detected in spermatophores of P. indicus (Muthuraman and K.G. Adiyodi, 1983). Spermatophores with wing-like processes have been also reported from Penaeus trisulcatus by Malek and Bawab (1971) and in some American white shrimps (Farfante, 1975).

Non-stalked spermatophores vary not only in their morphology but also in their mode and place of origin. In many Brachyura, for example, spermatophore formation is generally completed much prior to ejaculation, viz. in the anterior vas deferens itself (Libinia emarginata; Hinsch and Walker, 1974; Paratelphusa hydrodromous: Mathad and K.G. Adiyodi, 1979; Cardisoma carnifex: Kalyanram and Chandran, 1982) or in the mid

vas deferens (Barytelphusa pulvinata: Hebbar, 1982; Gecarcinucus steniops: Varghese, 1984). The number of spermatophores produced is generally large in these species, but the number of spermatozoa per spermatophore is small. In Dolops ranarum (Branchiura), the spermatophores are formed only during ejaculation and conclusive shape is attained only externally (Fryer, 1960). In the male system, therefore, the spermatophores cannot be seen in their final form.

The precise mode of formation of non-stalked spermatophores is not clearly understood in most species. Initially, spermatozoa from the testis are transferred to the vas deferens but the mechanisms of spermiation are little understood. Wolfe (1971) claimed that movement of the spermatozoa out of the testis is effected by "extra-testicular activity" i.e. by action of the dorsal and ventral longitudinal muscle bundles. General body movement is considered to be responsible for the compression of the testis, which forces its contents into the vas deferens. According to Malek and Bawab (1974), muscular movements of the vas deferens are responsible for the conduction of the spermatozoa. However, Matthews (1954a, b, 1957) thinks spermatozoa are moved passively by the pushing action of the developing spermatozoa.

In Libinia emarginata, the spermatozoa are grouped into spermatophores in the region of the anterior vas deferens; spermatophores are produced even before the crab attains its full size (Hinsch and Walker, 1974). In Penaeus kerathurus, the spermatozoa are aggregated to form coherent masses in proximal regions of the vas deferens. A secretion from the anterior region of vas deferens seems to aid as an adhesive substance in this process (Malek and Bawab, 1974). Towards the posterior region of the vas deferens, the major layers of spermatophore walls are deposited, partially around the sperm mass and partially as independent solid accessory wing. In addition, protective layers are added to the spermatophore in terminal ampoule, which is located towards the distal end of the vas deferens. In Cancer, the spermatozoa are packed into spermatophores by condensation of a secretion produced by the innermost layer of the epithelial cells of the proximal vas deferens (Langreth, 1969). The primary spermatophore wall in lobsters is secreted by certain highly coiled tubes that run both anteriorwards and posteriorwards in testis. As it moves down the vas deferens, spermatophore wall gets thickened and maximum thickness is attained as it reaches proximal vas deferens (Aiken and Waddy, 1981). In Hawaiian red lobster Enoplometopus occidentalis, spermatophore formation commences in the

coiled proximal region of the vas deferens (Haley, 1984). In Porcellio scaber, the spermatozoa in sperm bundles or spermatophores are cemented by secretions rich in acid mucopolysaccharides, produced by certain "giant cells" distributed along the walls of the vas deferens (Newstead and Dronfield, 1965). In Libinia emarginata, electron-microscopic studies have revealed that the spermatozoa are surrounded by a flocculent material in anterior regions of the vas deferens; posteriorly, a granular electron-dense secretion appears to separate them into clumps. The fluid surrounding spermatozoa showed an intensely positive reaction to both bromophenol blue and alcian blue; spermatophore walls also showed corresponding reactions. The single-layered spermatophore wall of L. emarginata is composed of a homogeneous, granular and medium electron-dense material (Hinsch and Walker, 1974). It may be recalled that spermatophore walls of insects and many crustaceans contain periodate-reactive and alcianophilic substances (K.G. Adiyodi and R.G. Adiyodi, 1975). The number of layers in spermatophore wall of crustacean varies with species. For instance, spermatophore wall in Penaeus setiferus is single layered (King, 1948), and in Scylla serrata two layered. In S. serrata, the outer wall is chitinous with a high content of sulphated acid mucopolysaccharides, and is penetrable

to vital dyes such as methylene blue, toluidine blue and basic fuchsin (Uma and Subramoniam, 1979). Chitinous spermatophore walls have been described also from Carcinus maenas (Spaulding, 1942), Tisbe holothuriae (Pochon-Masson and Gharagozlou-van-Ginneken, 1977), and Cardisoma carnifex (Kalyanram and Chandran, 1982). In Enoplometopus occidentalis (Nephropidae), spermatophore has an inner (PAS-positive) and an outer (PAS-negative) layer; the outer layer forms a broad cap on one side (Haley, 1984). Spermatophore walls are triple layered in Panulirus homarus (Berry, 1970), Portunus (Ryan, 1967), and Pacifastacus leniusculus (Dudenhause and Talbot, 1983). In Penaeus trisulcatus, the spermatophore wall is said to be comprised of as many as seven layers (Malek and Bawab, 1971).

Spermatophore of Dolops ranarum is unique among crustaceans in many respects. It is produced by a special gland of the male called spermatophoric gland (Fryer, 1960). The gland is comprised of a system of branched ducts lined by glandular cells whose cytoplasm and nuclei stain deeply with haematoxylin. The spermatophoric canals formed by union of collecting ducts contain a whitish secretion which shows staining reactions similar to those of spermatophore walls. This secretion is very sticky and fairly elastic; when it

comes into contact with water some chemical changes followed by colour change take place and it becomes hard. The spermatophoric canals lead to a spermatophoric vesicle into which the vasa deferentia also open. Sperm mass from the vas deferens enters the spermatophoric vesicle as a result of peristaltic movements of the vas deferens and associated tissues. Here the sperm mass is completely coated with the whitish secretion produced by spermatophoric gland cells. The contents of both spermatophoric vesicles are ejected together at the time of mating. The exudates fuse to form a single spermatophore, which is pushed against the female. Spermathecal spines pierce the pliable walls of the spermatophore leaving two apertures. The spermatophore wall molds itself to the contours of the cuticle of the female and remains attached to it. The fully formed spermatophore is globular and has a pearly appearance. The walls do not contain chitin (soluble in hot KOH) and are basically proteinaceous, and the hardening of the wall appears to be due to quinone-tanning. The mechanism of hardening of spermatophore walls is not understood in most crustaceans, but there is no denying that hardening occurs in some species. In Penaeus kerathurus, only the lipoprotein layers in spermatophore wall show changes leading to hardening. Phenolic groups and a phenolase seem to be involved in this process

(Malek and Bawab, 1971). Hardening of spermatophores, following extrusion from the male has been reported also in the crayfish, Pacifastacus leniusculus (Dudenhausem and Talbot, 1983).

In Squilla holoschista (Stomatopoda), unlike many other Malacostraca, the spermatozoa are not packed into discrete spermatophores, but are concentrated to form a sperm cord. Spermatozoa are stored in mid vas deferens; in the distal vas deferens, the spermatozoa undergo much reduction in size and are organized into sperm cord. A non-sulphated mucopolysaccharide secretion of the distal vas deferens helps in formation of the sperm cord (Deecaraman and Subramoniam, 1980).

1.4. SEMENOGENESIS

In crustaceans, the seminal plasma is generally the pooled product of gland cells distributed in the vas deferens itself; in some species, products of special collateral accessory glands also contribute to its formation. The pattern of distribution of gland cells in sperm duct varies between species and within the same species along the sperm duct. In Libinia emarginata, for example, the more posterior parts of vas deferens are involved in production of seminal fluid, while in the anterior vas deferens (AVD), the

spermatophores are formed. The walls of AVD of this species are lined with columnar cells with large nuclei (Hinsch and Walker, 1974). Greenwood (1972) recognized seven functionally different regions in vas deferens of Paqurus novae-zealandiae. Mathad and K.G. Adiyodi (1979) distinguished three regions in vas deferens of Paratelphusa hydrodromous, each with a distinct role in male reproductive physiology. In Homarus (Herrick, 1909) and Nephrops norvegicus (Farmer, 1974), the vasa deferentia are composed of three distinct regions: (1) a short opaque proximal portion that serves to conduct sperm mass from testis, (2) an elongated coiled semitransparent section which secretes a gelatinous matrix and (3) a muscular ejaculatory duct. The vas deferens of Chionoecetes opilio and C. bairdi has 3 regions - spermatophorogenous tubule, spermatophorotheca, and rosula (Sapelkin and Fedoseev, 1981). In some decapods, regional differentiation of the vas deferens is so prominent that even a separate semen storage organ - seminal vesicle - can be recognized. In Penaeus kerathurus, three different regions of the vas deferens are concerned with varying stages of spermatophore formation, and the final stage is attained in the terminal ampoule (Malek and Bawab, 1974).

Epithelial lining of the vas deferens of Emerita asiatica produces 2 types of secretions; one is used for the formation of spermatophore and the other is used as the gelatinous embedding matrix (Subramoniam, 1981). In Albunea symnisia, the vas deferens is a straight tube having ascending and descending limbs. The epithelial cells lining the ventral and dorsal periphery of the lumen produce secretions used in formation of the spermatophore, connecting cord and putty-like gelatinous matrix (Subramoniam, 1981). In Scylla serrata, ascorbic acid present in high concentration in the seminal plasma appears to be secreted by the testis and the proximal vas deferens (Uma and Subramoniam, 1981a). The testis and proximal vas deferens are also said to be involved in production of protein components which go into the formation of spermatophores and seminal plasma, whereas mucous products in seminal plasma are elaborated by epithelial cells of the mid vas deferens. A possible transfer of blood proteins into the vas deferens is indicated by electrophoretic investigations by Uma and Subramoniam (1981b), but more critical work is required to verify this claim.

In some species of crustaceans special structures are developed by the vas deferens to increase the secretory surface. Thus, Matthews (1951) described in Panulirus

a typhlosole-like formation which projects from the "hyaline-line" into the lumen and secretes the putty-like matrix of the spermatophoric mass. Vas deferens of Jasus novaehollandiae shows a similar typhlosole-like formation (Fielder, 1964). In the vas deferens of Penaeus indicus the typhlosole-like structure extends as far down as the seminal vesicle (Muthuraman and K.G.Adiyodi, 1980). The distal vas deferens of Squilla holoschista has four ciliated typhlosole-like structures formed by modification of the inner epithelial layer (Deecaraman and Subramoniam, 1980). However, in Artemia the vas deferens gives almost the same histological picture throughout its length, i.e., two external muscular layers and an internal layer of squamous epithelial cells (Wolfe, 1971). The only difference noted was the increased thickness of the muscular layers towards the posterior end. In Artemia, the whole vas deferens appears to be involved in storage of spermatozoa and secretion of the seminal fluid.

From the foregoing account, it is obvious that the sperm duct in crustaceans shows varying degrees of specialization for secretion of the seminal plasma and spermatophore wall material and for storage of the spermatozoa. The gland cells in sperm ducts responsible for production of specific constituents of the seminal

plasma and spermatophore wall material have not been precisely mapped; all we know at present is that the cells in a particular region may be involved in production of seminal plasma as a whole or spermatophore wall material. In species showing extreme regional specialization in vas deferens, it is possible different components are secreted by different types of gland cells distributed in a 'logical' sequence along the sperm duct; in species with less specialized vas deferens (e.g. Artemia), the gland cells also may be less specialized and secrete a range of products.

1.5. ACCESSORY SEX GLANDS AND THEIR FUNCTIONS

The secretion of the sperm duct of many insects and of some crustaceans is supplemented with secretions of certain well-defined accessory sex glands (ASG) which are essential for reproduction. Based largely on locational morphology of glandular elements in relation to the genital system, K.G. Adiyodi and R.G. Adiyodi (1975) have classified ASG into different types: gonadal, ductal, seminal, spermathecal, genitoatrial, penial, male collateral and female collateral, but such distinctions are rendered difficult in some species. The structure and function of accessory sex glands in insects have been extensively studied and recently reviewed by

Happ (1984) and Gillott (1985). We shall give here only a few examples, mostly of male collateral glands. Of the various tubules present in mushroom-shaped ASG of Periplaneta americana, the utriculi majores are the largest. They are lined by a single layer of secretory cells with basal plasmalemmal infoldings and apical microvilli and surrounded by a coat of muscle. Glandular epithelial cells have an extensively developed rough endoplasmic reticulum and active Golgi complex, sub-cellular features suggestive of synthesis and transport of proteins. The secretory product of utriculi majores is rich in protein, neutral lipids and phospholipids (R.G. Adiyodi and K.G. Adiyodi, 1974). The merocrine cells of the inner pair of ASG in Hydrophilus olivaceus produce a muco- or glycoprotein with little RNA component, whereas the outer pair of massive glands produces a muco- or glycoproteinaceous secretion rich in RNA (Gundevia and Ramamurty, 1977). In Poecilocera picta, each lobe of the ASG has twelve tubules which can be classed into two groups - one containing a whitish dense secretion and the other containing a colourless secretion; the products of different tubules mix just prior to the formation of the spermatophore (Veera Raghavan et al., 1982). ASG of the Colorado beetle Leptinotarsa decemlineata secretes at least fifty polypeptides of varying molecular weights. Apart from autosynthesis,

these glands also take up protein from the haemolymph (Peferoen and DeLoof, 1983, 1984a, b), reminiscent of hetro-synthesis and uptake of vitellogenin in female insects. In the locust Locusta migratoria migratoriodes, ASG secretions are mainly proteins having a wide range of molecular weight and charge properties (Lange and Loughton, 1984). In vitro studies on the rate of protein synthesis in the ASG of Drosophila melanogaster show that the rate is higher in mated males than in unmated males (Perrin-Waldemer and Stepien, 1984, 1985).

Among Crustacea, the presence of discrete ASG has been reported only in a few cases. In Artemia, for example, a pair of ASG is present toward the tip of the eversible penis and can be called penial glands, according to the terminology adopted by K.G. Adiyodi and R.G. Adiyodi (1975). The gland consists of approximately twenty pairs of gland cells. It is comparable in origin and structure to the shell gland of females. The secretion is composed of either neutral proteins or mucoproteins (Wolfe, 1971). Secretions of this gland may (1) aid in lubrication of uterus and thus facilitate intromission, (2) activate spermatozoa and/or (3) help in formation of the sperm plug in females. Accessory gland cells have been reported also in seminal vesicles of mysids (Kasaoka, 1974). The secretion which may be used to nourish the

spermatozoa is added to the spermatophore complex in the region of the seminal vesicle. Discrete male ASG have been reported from Ocypode ceratophthalmus (Sreenarayan and R.G. Adiyodi, 1979), Squilla holoschista (Deecaraman and Subramoniam, 1980) and Cardisoma carnifex (Kalyanram and Chandran, 1980). More recently, Haley (1984) has reported the presence of a tubular gland in terminal portion of the vas deferens of Enoplometopus occidentalis.

Functional significance of male ASG varies in different species among arthropods. In many insects, these glands aid in production of various components of semen. In Gomphocerus rufus and Glossina morsitans morsitans, ASG secretion is used in formation of the spermatophores. In G. rufus, the various tubules of ASG are concerned with various functions such as determination of the shape of the spermatophore, transmission of pressure necessary for its transfer, and formation of lumen in the spermatophore (Hartmann, 1971). ASG secretions of Glossina are neutral acid mucopolysaccharide-protein complex, which go into the formation of spermatophores (Odhiambo et al., 1983). In houseflies and culicine mosquitoes, male ASG secretion passed out during copulation induces loss of female sexual receptivity and promote oviposition (see Riemann, 1973 and Gillott

and Friedel, 1977 for references). In Aedes, male ASG produce a substance called matrone (Hiss and Fuchs, 1972). Matrone, purified and administered into virgin females, prevented insemination and stimulated egg-laying. Implantation of ASG into virgin females induced oviposition in Schistocerca (Leachy, 1973b), Melanoplus (Pickford et al., 1969) and Stomoxys calcitrans (Morrison et al., 1982). According to Leachy (1973a), copulation with males without ASG resulted in a fall in fecundity suggesting that active secretion of male ASG is required to effect a high level of fecundity. ASG secretion enhanced egg maturation as well. In many insects (mostly Diptera) ASG produce fecundity-enhancing and receptivity-inhibiting substances (see Gillott and Friedel, 1977). In insects, ASG are also believed to aid in vitellogenesis (Friedel and Gilliott, 1977), induce contraction of the oviduct (Devey, 1958) and ensure successful coupling and normal coupling time (Errard, 1978). In ticks male ASG secretions induced maturation of spermatozoa in female reproductive system (Shepherd et al., 1982). Recent studies by Loher (1984) in crickets have shown that prostaglandin E_2 (PGE_2) produced by spermatheca of females serves as an oviposition stimulus; the enzyme necessary for biosynthesis of PGE_2 in spermatheca is transferred to the female via the spermatophore. Interestingly, in some cockroaches, ASG acts as storage excretory organs

(Roth and Dateo, 1965). Large amounts of uric acid are stored and excreted during copulation, thus protecting the spermatophores.

Among crustaceans, in Dolops ranarum, the ASG is essential for spermatophore formation (Wolfe, 1971). The club shaped ASG of Squilla holoschista open separately at the tip of the intromittant organ. Their secretion, rich in protein and neutral mucopolysaccharide, is suggested to aid (1) in dissolving the cementing substance of the sperm cord in oviduct (Deecaraman and Subramoniam, 1980) and (2) in vitellogenesis (Deecaraman and Subramoniam, 1983). Kalayanram and Chandran (1982) think the secretion of ASG of Cardisoma carnifex may dissolve spermatophore walls in the region of the posterior vas deferens, but no experimental evidence has been furnished to substantiate this suggestion. In Enoplometopus occidentalis, the tubular gland produces an extra-spermatophoral gelatinous secretion (Haley, 1984).

1.6. SPERMATOGENESIS AND SPERM FUNCTION

Spermatogenesis and sperm function in crustaceans have been reviewed recently by Pochon-Masson (1983). Morphology of the testis varies widely in different groups of Crustacea. In lobsters, for example, the testis is a recemose or compound gland consisting of collecting

duct and smaller tubules that terminate in acini (Matthews, 1951). In the prawn Penaeus indicus (Natantia), the testis is pentalobulate (Muthuraman and K.G. Adiyodi, 1980), whereas in many brachyurans it is a simple, narrow and elongated structure.

Testicular chemistry of crustaceans has not been studied in any great detail in relation to spermatogenesis. In our laboratory, investigations by Mathad (1983) on the crab Paratelphusa hydrodromous have shown that the testis is rich in basic proteins and lipids. Appreciable quantities of Millon-positive material, components containing 1:2 glycol groups, and ascorbic acid have been detected in testis of P. hydrodromous. In Scylla serrata, testis contains large quantities of ascorbic acid and the bulk of the ascorbic acid in seminal plasma of this species is suggested to be of testicular origin (Uma and Subramoniam, 1981a). In Penaeus indicus, the testis contains proteins ($11.55 \pm 0.68\%$), oligosaccharide fraction ($1.72 \pm 0.09\%$) polysaccharides ($1.59 \pm 0.24\%$) and reducing sugars ($0.49 \pm 0.02\%$) (Muthuraman and K.G. Adiyodi, 1983). We think the organic constituents present in testis may serve as fuels for spermatogenesis and for production of testicular secretions and perhaps also as reserves to be utilized in times of need. Little is known of the metabolic pathways used by testis to

derive energy in crustaceans. The testis of Carcinus maenas has some of the enzymes (e.g. 17 β -hydroxysteroid oxidoreductase) necessary for steroid bioconversions; testis of this species has also the ability to convert androstenedione to testosterone (Blanchet et al., 1972). Homogenates of testis from the spiny lobster, Panulirus argus show appreciable degree of ecdysone mono-oxygenase activity (James and Shiverick, 1984). The testis of Homarus americanus contains 20-ketone reductase capable of converting $[^{14}\text{C}]$ progesterone to 20 α -dihydroprogesterone. The testes of this species can also convert $[^{14}\text{C}]$ pregnenolone to 20-dihydropregnenolone (Burns et al., 1984a). Homarus testis contained 14.3 mg/g of ~~testosterone~~ testosterone Burns et al., 1984b).

In Crustacea, the various processes ultimately leading to the production of male gametes (spermatidogenesis and spermiogenesis) are normally completed in the testis itself. In Macrobrachium idella, a number of protogonia are seen along the wall of testicular acini, sandwiched between mesoderm cells. Protogonia grow in size to form primary spermatogonia. Each of the primary spermatogonia is separated from its neighbours by a row of mesoderm cells. With increase in number of primary spermatogonia changes appear in pattern of their arrangement within the acinus, each cluster of primary spermatogonia now

being surrounded by mesoderm cells. The secondary spermatogonia are smaller than the primary spermatogonia. The nucleolus, present in primary spermatogonia, is not visible in secondary spermatogonia. The primary spermatocytes are generally more basophilic with only small quantities of cytoplasm, the nucleus nearly filling the whole cell. Secondary spermatocytes of Macrobrachium idella are generally eosinophilic. Conical spermatids undergo spermiogenesis and give rise to mushroom-shaped spermatozoa (Sreekumar et al., 1980; Sreekumar and R.G. Adiyodi, 1981, Sreekumar, 1985).

The process of spermiogenesis has been studied in different species of Crustacea (see Pochon-Masson, 1983 and R.G. Adiyodi, 1985 for review). In Tisbe holothuriae for example, spermiogenesis is completed in 3 stages (Pochon-Masson and Gharagozlouvan Ginneken, 1977). Initially, the spermatids are round in shape. The chromatin exhibits an anterior polarity and the nucleolus disappears. The cytoplasm has many ribosomes, a mitochondria, Golgi apparatus and large cisternae lying parallel to the cell membrans. In the second stage, the cell elongates and nucleus moves to the anterior end. The peripheral cisternae are converted to an axial highly osmiophilic sheet. Two long mitochondria are disposed symmetrically. In the hyaloplasm some longitudinal

microtubules appear. Finally, the cell elongates further, resulting in the elimination of the posterior cytoplasmic mass, shedding the two mitochondria. In Emerita analoga, the developing spermatids lose a significant proportion of their nuclear protein - mainly the somatic type of histones during spermiogenesis with the result that in late spermatids nuclear histones and proteins are almost absent. It has been also observed that the non-basic acrosomal proteins are first synthesized in cytoplasm and subsequently transferred to the acrosome (Vaughn and Thomson, 1972). In Asellus aquaticus, the nuclei of almost all cellular stages in spermatogenesis contain non-histamic proteins. In this isopod, the rate of incorporation of lysine and arginine into germ cells varies in different stages of spermatogenesis; it is very small in spermatogonial metaphase and anaphase^{and} is almost nil in meiotic metaphase -I. The synthesis of non-histamic proteins declines during maturation of the spermtid and ceases in the sperm (Brasiello, 1971).

Variations in structure of crustacean spermatozoa have been recognized for a long time (see Pochon-Masson, 1968, 1983; Baccetti, 1969). But information on metabolic demands and physiological activity of crustacean spermatozoa is far from impressive. In Balanus balanus, the spermatozoa themselves contain adequate amounts of glycolysable

substances so that the spermatozoa can maintain their metabolism for long periods. The rate of respiration of these spermatozoa is reportedly low; the low O₂ uptake appears to be correlated with their low motility and low ATP content (Barnes, 1962b).

Sperm-egg interaction has been studied only in a few crustaceans. Spermatozoa of crustaceans are primarily atypical and non-flagellated. Barring a few exceptions (cirripedes, ostracods and mystacocarids), crustacean spermatozoa are immotile. Decapod spermatozoa exhibit a peculiar 'acrosomal reaction'. In Callinectes sapidus, for example, 'explosion' of spermatozoa occurs in seawater suspension due to osmotic shock. Prior to explosion, the spermatozoa become more spherical and the acrosomal region elongates. The rays become shortened. Then a pronounced eversion of the acrosome (capsule or tail) is seen. The acrosomal region projects out giving the appearance of a spike (Brown, 1966). In Libinia emarginata, acrosomal reaction consists of a rapid elongation of the acrosome, drawing up of the radial arms and a loss of refringence of the acrosome. It is followed by eversion of the acrosomal tubule (Hinsch, 1971). The acrosomal reaction in Carcinus maenas also follows the usual Reptantia Decapoda pattern (Goudeau, 1982). Although these processes are slow and gradual under normal spawning

conditions, similar reactions could be elicited resulting in fusion of the apical cap with the surface of egg coat and extrusion of the acrosomal contents. These reactions are considered to represent the final preparation of the spermatozoa for fertilization (Hallez, 1874; Labbe, 1904). Many experimental studies have attempted to induce eversion by treatment of the spermatozoa with chemical solutions and ovarian fluids and by varying the mechanical and osmotic pressure. Herrick (1909) mentions 4.2% isotonic solution of calcium chloride in seawater as effective for spermatozoa of Homarus, while Talbot and Chanmanon (1980) have reported success with calcium-transporting ionophore A23187. On natantian spermatozoa, a comparable observation has been made more recently by Clark et al. (1981). In the freshwater prawn Macrobrachium rosenbergii, mature spermatozoa resemble everted umbrella with a spike projecting perpendicularly from the base of the convex surface. In this species, during fertilization, base of the spermatozoon attaches first to the investment of egg; then the spike bends at the base and contacts the investment (Lynn and Clark, 1983a). There is scope to use artificially induced acrosome reaction as a test of the fertilizability (viability) of the spermatozoon. It would be of great interest to check in different species at what point along their sojourn down the sperm duct the spermatozoa acquire the ability to fertilize eggs and

whether some capacitation is needed for the spermatozoa to be effective in fertilization.

1.7. FATE OF SEMEN IN THE FEMALE SYSTEM

Semen, transferred to the female during copulation, undergo certain structural and biochemical changes while within the female system. A secretion (glair) in female Pacifastacus trarbridgii serves to dissolve the spermatophore walls (Masson, 1970). Seminal vesicle of Balanus balanus contains a mixture of immature (with a thickened region just behind acrosome) and mature (having no such thickened region) gametes in varying proportions (Walley et al., 1971). In mantle cavity of a receptive individual, before oviposition, they exhibit only low motility. However, if secretion from oviducal gland is added, vigorously swimming spermatozoa are liberated from the sperm mass. Not only mature but also immature spermatozoa become motile. The secretion from oviducal gland of B. balanus is suspected to contain some sperm-activating factors. In Cypridopsis sp., the filiform spermatozoa are surrounded by an outer sheath while the spermatozoa are in the male genital ducts, but such a sheath is absent in spermatozoa in female genital ducts. Spermatozoa from female genital duct alone were motile (Reger and Florendo, 1969^{a,b}). In Tetranychus urticae (Acari), a change in shape and size of the spermatozoa

occurs after reaching the receptaculum seminis of female (Mothes and Seitz, 1981). In many arthropods the semen is stored, after copulation, in the spermatheca of females. In such storage organs the spermatozoa are kept viable for periods ranging from weeks to months. A gradual reduction in viability (evidenced by fertilizing ability) of spermatozoa, however, occurs in the spermatheca, with advancing period of storage (Paul, 1984). Though the actual mechanism by which spermatozoa are kept viable for long periods in crustaceans is not known, the ability of spermatozoa to survive under conditions of high sperm density and low oxygen tension has been reported long back. The low oxygen uptake and lack of motility of Balanus balanus spermatozoa have been attributed to the relatively low level of respiratory enzyme system present in them (Barnes, 1962b). Spermatozoa may derive their energy from endogenous sources. Taber (1977) reported that storage of spermatozoa over long duration in spermatheca needs rapid fructolysis and Apis sperm is rich in fructose. In sea urchins, the seminal plasma contains little exogenous substrate capable of maintaining the metabolism of spermatozoa. Sea urchin spermatozoa utilize, therefore, endogenous substrates, mainly phospholipids (Rothschild and Cleland, 1952). According to Anderson and Personne (1970), spermatozoan survival depends probably on anaerobic utilization of endogenous substrates, and they described glycogen (localized in spermatozoa) as an

important endogenous energy source. However, in many cases, either endogenous substrates are too small or are not utilized till about the time of fertilization, making it mandatory that the spermatozoa depend on extracellular metabolites for survival during storage in spermatheca. Grodner (1979) reported in the insect Anthonomus grandis that the spermathecal walls secrete some substance which is nutritive to spermatozoa, and similar observations have been made by Krishnakumar (1985) in the crab, Paratelphusa hydrodromous.

1.8. REPRODUCTIVE CYCLICITY AND ROLE OF EXOGENOUS FACTORS

As in many other invertebrates, reproductive activity in both sexes of crustaceans is closely linked to environmental factors (K.G. Adiyodi and R.G. Adiyodi, 1970). In Menippe mercenaria, for example, high temperature can induce spawning and the seasonal dependence of spawning is believed to be due to variations in seasonal temperature (Cheung, 1969). Prolonged starvation (early in season) inhibits gametogenesis and fertilization in Semibalanus(Balanus)balanoids (Barnes and Barnes, 1967). Starvation leads to a suppression of reproductive activity in female Paratelphusa hydrodromous, and reserves meant for reproduction are channelled to maintain somatic metabolism (R.G. Adiyodi, 1969). In P. hydrodromous,

the ovarian cycle is intimately linked to monsoons (i.e. availability of water) (Anilkumar, 1980), and the spermatogenic activity is seasonal (Suma, unpublished).

In several Crustacea, seasonal variations in environment are utilized to trigger molting and reproduction either as synchronous events (Decapoda, Natantia, Amphipoda) or as asynchronous events (Brachyura). Temperature and photoperiod act on endocrine centres, thereby regulating seasonally dependent moulting and reproduction (Aiken and Waddy, 1981). Temperature plays a critical role in reproduction of copepods also (Watras, 1983). In Homarus americanus, temperature influences the size of the vas deferens: males exposed to 15°C have a much larger 'vas deferens factor' than control males kept at ambient temperature (Aiken and Waddy, 1981). Though breeding can take place throughout the year, Black (1966) reported two peaks of sperm production in crayfish. In the barnacle Semibalanus (Balanus) balanoids, breeding is controlled by temperature and light. A temperature below the critical value of 10°C and a light periodicity less than 12 hours/day are necessary for normal breeding activity (Tighe-Ford, 1967). In Uca pugnax, a short photoperiod prevents spermiogenesis; too much illumination also has an adverse effect. Furthermore, spermatogenic activity is cyclic in this species although experimental alteration of photoperiod

cannot induce spermatogenesis out of season (Young, 1974). In the freshwater crab Potamon kooloonese also spermatogenic activity is seasonal (Joshi and Khanna, 1982). Oocyte size of Orconectes nais is negatively correlated with photoperiod. Here day length influences ovarian growth by partitioning of energy between hepatopancreas and ovaries (Rice and Armitage, 1974). Breeding activity of the isopod Asellus aquaticus shows correlation with the length of day-light and annual temperature changes (Strus and Blejec, 1983). Vitellogenesis in Porcellio dilatatus (Isopoda) also shows a diurnal rhythm (Gohar and Souty-Grosset, 1984). Studies by Nelson et al. (1983) show that in lobsters final vitellogenesis is initiated only during the months between vernal and autumnal equinoxes. In Oziotelphusa senex senex (= Paratelphusa hydrodromous), there is no correlation between breeding cycles and environmental factors such as temperature and photoperiod; in this freshwater crab availability of water and food are considered as the major factors determining the breeding period (Ramamurti et al., 1983) which confirms the observations of Anilkumar (1980). Even in the same general locality, crabs of the same genus may exhibit different patterns of annual breeding cycle, which is attributed to differences in the immediate environmental conditions Chandran et al., 1982). Conversely, Ocypode rotundatus showed the same type of

reproductive rhythm in two different localities (Chandran et al., 1981). Reaka (1976) has reported that the moulting and reproduction in stomatopod crustacea are influenced by tidal and lunar periodicity. Uca pugnax exhibits a semi-monthly reproductive cycle (Greenspan, 1982).

The mating time and breeding period of P. hydrodromous varies according to the size of the crab and climatic variations (Anilkumar, 1980). In Barytelphusa guerini, annual reproductive cycle is divided into reproductive, postreproductive and prereproductive periods (Gangotri, 1971). Joel and Sanjevaraj (1982) reported that Scylla serrata and Scylla transquebarica showed two peak breeding seasons, one in summer when temperature and salinity are high and the other in monsoon when both salinity and temperature are low. Glycogen and lipid content of the testis in Scylla serrata increased during the peak reproductive period (Farooqui and Nagabhushanam, 1983). A comparable observation has been made in Macrobrachium kistnensis also (Mirajkar et al., 1983). In Uca annulipes, Portunus pelagicus and Metapenaeus affinis, breeding season extends for several months with peak periods of gonadal activity (Pillai and Nair, 1971). In Penaeus indicus, during April-June, major biochemical components of the vas deferens and seminal vesicles are appreciably more than during January-March (Muthuraman and K.G. Adiyodi, 1981).

1.9. ENDOGENOUS FACTORS INFLUENCING REPRODUCTION

Moulting cycle and ovarian cycle proceed synchronously or in mutual exclusion in Malacostraca (R.G. Adiyodi, 1985)

for review). It would be interesting to examine whether a similar relationship exists between moulting on the one hand and spermatogenesis along with the semen production on the other. Studies in our laboratory have shown that in Macrobrachium idella (Sreekumar and R.G. Adiyodi, 1981) and Penaeus indicus (Muthuraman, 1985) these processes are so balanced that during the reproductive season a specific stage in spermatogenesis and seminal plasma production corresponds with a specific stage in the moulting cycle. An interplay of hormones of the kind involved in control of moulting and reproduction of females may perhaps be expected to be involved in control of moulting and reproduction in males; but the males additionally have androgenic hormone (absent in females) and females have ovarian and vitellogenin-stimulating ovarian hormones which are absent in males.

1.9.1. Role of Neurohormones

Information is rather limited on the endocrine control of reproduction in male crustaceans. Targets of hormones influencing reproductive activity of males are the testis, genital ducts and accessory sex glands (ASG) and may also include the central nervous system by which male sexual behaviour is produced. Physiological activity of ASG in crustaceans appears to be under the control of gonadotropins and/or androgenic sex hormones.

Activity of ASG of some insect species has been clearly shown to be influenced by juvenile hormone (JH) produced by the corpora allata. For example, extra production of JH in monarch butterflies leads to posteclosive enlargement of the ASG in males (Herman, 1975). In Diptera, Coleoptera and Lepidoptera, JH is reported to regulate the activity of ASG. In cockroaches, accumulation of structural protein of the ootheca and of protocatechuic acid glucoside in left collateral glands is under the influence of JH (K.G. Adiyodi and R.G. Adiyodi, 1974 for review). Protein synthesis of the transparent accessory reproductive gland of male Rhodnius prolixus is controlled directly or indirectly by the neuroendocrine system (Barker and Davey, 1981). Allatectomy conducted at the time of emergence of adult desert locusts led to underdevelopment of secretory apparatus of the ASG. The maturation of all glands (except gland 16) is under the control of JH (Odhiambo, 1971). The development of male ASG in Melanoplus sanguinipes is also controlled by JH. However, the response of different gland tubules to JH replacement therapy is varied. The synthesis of some proteins of ASG of this insect is regulated with JH, whereas the production of other proteins is suspected to be JH-independent (Gillott et al., 1984). Ecdysteroids appear to be required for differentiation of ASG in insects. Transplantation experiments using male ASG of Pleberioeryllus

guttiventris have shown that the differentiation of the gland tubules takes place when MH titre is high and JH is absent (Ranganathan, 1977). In Dysdercus similis, the volume of ASG changes in accordance with its activity. A JH analogue brought about initial hyperactivity of these glands, followed by gradual degeneration (Judson, 1981).

Among crustaceans, in Paratelphusa hydrodromous, secretory activity of the vas deferens epithelial cells which is seasonal has been demonstrated to be under the inhibitory control of eyestalk principles (Mathad and K.G. Adiyodi, 1981). R.G. Adiyodi (1985) has further shown that seasonal changes take place in androgenic gland activity of this species, suggesting the possibility that androgenic hormone may be involved in some way in this process. As it is difficult to remove surgically androgenic gland from crabs and pure preparations of the androgenic hormone are not available for testing, it will be some time before this problem can be settled.

In the male prawn Penaeus japonicus withdrawal of eyestalk principles resulted in a remarkable development of the sperm ducts. This precocious development of sperm ducts could be inhibited by a heated aqueous extractable factor taken from the eyestalks of Penaeus vannamei (Chim et al., 1983).

Control mechanisms involved in spermatogenic activity of crustaceans are only beginning to be understood. Ōtsu (1963) found that development of the testis is hastened by removal of the eyestalks. Gonadal development, according to Ōtsu (1963), is controlled by two antagonistic factors, viz. a suppressing factor from eyestalks and a stimulating factor from the thoracic ganglion. In the freshwater crab Paratelphusa hydrodromous, transplantation of thoracic ganglia or brain led to a notable development of the testis and of the vas deferens (Gomez, 1965). Demusy (1953) found that eyestalk extirpation in immature crabs resulted in precocious spermatogenesis in Carcinus maenas. The inhibiting effect by eyestalk extracts on testicular development has been reported also in Parapenaeopsis hardwickii (Kulkarni and Nagabhushanam, 1984). In the crayfish Pontastacus leptodactylus, gametogenesis is believed to be inhibited by a mesodermal tissue surrounding the primary gonidia (Amato and Payen, 1978). The primary gonidia are transformed into secondary gonidia only after the withdrawal of the mesodermal tissue which could be experimentally induced by surgical removal of the eyestalks. In P. leptodactylus, the secondary gonidia show a higher level of RNA synthesis which is followed by meiosis. This usually takes place during summer. Androgenic hormone is reported to play a major role in this process. In male gonochoristic shrimps (Leander

serratus and Crangon crangon), the median protocerebrum produces a hormone necessary for maintenance of: (1) the spermatogonia, spermatogenesis and mesoderm cells of the testis, (2) the epithelium of spermduct and (3) the androgenic glands (Touir, 1977a, b).

The gonad-inhibiting hormone (GIH) produced by eyestalks and the gonad-stimulating hormone (GSH) produced by brain and thoracic ganglia are both presumed to be protein or polypeptide (see K.G. Adiyodi and R.G. Adiyodi, 1970 for details). Recently, GIH and the moult-inhibiting hormone (MIH) have been separated from eyestalks of the lobster Panulirus argus. Partial characterisation of GIH has been achieved and its molecular weight is said to be about 5000 daltons (Quackenbush and Herrnkind, 1983). According to Bomirski et al. (1981) GIH is neither sex- or species- specific; in Crangon crangon GIH is thermostable having a molecular weight of 2,000.

1.9.2. Androgenic Glands and Androgenic Hormones

Charniaux-cotton (1954, 1970, 1975) and her collaborators have made extensive studies on the role of the androgenic gland on sexual differentiation in amphipods. Removal of the androgenic gland leads not only to an inhibition of spermatogenesis, but also

to destruction of the testis and gradual loss of the secondary sex characters. Blood plasma of male Carcinus can induce development of male characters in female Orchestia; androgenic hormone, therefore, does not appear to be species specific (Charniaux-Cotton and Kleinholz, 1964). In the isopod Armadillidium vulgare, implantation of androgenic glands or injection of active extract of the androgenic glands from mature males to young females induced masculinization of the latter (Katakura and Hasegawa, 1983; Hasegawa and Katakura, 1983). Blanchet et al. (1972) consider testis and vas deferens as two target tissues of the androgenic hormone. There may be more. The chemical nature of the androgenic hormone is disputed. Berreur-Bonnenfant et al. (1973) consider the hormone as a lipoid molecule. Ferezou et al. (1977) reported the presence of two lipid methylesters in androgenic hormone of Carcinus maenas; androgenic gland has the ability to synthesize farnesyl acetone (E,E) (Ferezou et al., 1978). Farnesyl acetone, isolated from the androgenic glands of Carcinus maenas, inhibits vitellogenesis (Berreur-Bonnenfant and Lawrence, 1984). Histological and ultrastructural studies, however, seem to suggest a proteinaceous or polypeptide nature for the hormone (King, 1964; Meusy, 1965). Katakura et al. (1975) claim that androgenic hormone is a protein with a molecular weight of 16,000.

Androgenic gland of the protandric hermaphrodite Pandalus platyceros develops from epithelium of the vas deferens (Hoffman, 1969). According to Hoffman (1969), the developmental histology of androgenic cells can be divided into six discrete stages. Initially, the cells are in the stage of proliferation. The cells have relatively small cytoplasmic volumes and small irregularly shaped nuclei. This stage is followed by a stage of cytoplasmic hypertrophy which occurs during the active male phase (proliferation of spermatogonia). The cells now become elongated and have flattened, long nuclei. The stage of vacuolization is third. During this stage, vacuolated cells with swollen oval nuclei showing less affinity towards basophilic stains are seen. In the next stage (stage of cellular break down), there is a total degeneration of the cytoplasm. The fifth stage is seen in males transforming into female, (transitional atrophy) and is characterised by pycnotic nuclei and large cytoplasmic inclusions. In the final stage - ghost stage - empty sheaths of androgenic cords alone are seen. During this stage, no traces of testis can be seen in this protandric shrimp.

In Ocypode platytarsis, the length and width of the androgenic glands vary according to the size of the animal. The small cells (10-11 μ m) of androgenic glands contain

dense cytoplasm and no vacuoles. The nuclei are spherical and have dense granular chromatin. The medium-sized cells contain vacuoles and large nuclei. The large cells (15 μm) have prominent vacuoles; sometimes the vacuoles are so large that the cells appear empty (Thampy and John, 1970). In androgenic gland cells of Porcellio scaber, changes occur in cell organelles in the course of the secretory cycle; these holocrine cells which undergo regression and destruction pass their contents into spaces of the haemocoel (Radu and Crăciun, 1976). Different modifications of the androgenic gland cells leading to their degeneration have been further described in Ocypode quadrata by Payen (1972).

The androgenic glands are under the influence of eyestalk principles not only in adults but in larval stages also. Payen et al. (1971) reported that eyestalk ablation in the zoea of the crab, Rhithropanopeus harrisii and in the megalopa of Callinectes sapidus resulted in hypertrophy of the androgenic glands. Glandular hypertrophy is characterized by hyperplasia and an increase in cell size at the light microscopic level. At the ultrastructural level, the gland cells show well developed granular ER, mitochondria grouped into juxtranuclear area, a rounding of the nuclei and increase in accumulation of electron-dense material in the intercellular spaces.

In Pandalus platyceros, RNA synthesis in androgenic gland cells increases after eyestalk ablation and after administration of exogenous β -ecdysone (Foulks and Hoffman, 1974). This rises the question whether the effects of eyestalk ablation on androgenic gland are mediated by β -ecdysone. We consider this unlikely: the effect of β -ecdysone observed on RNA-synthesis in androgenic glands may be only the general effect of β -ecdysone to stimulate RNA synthesis in a variety of tissues, and furthermore, there is as yet no conclusive evidence to link RNA-synthesis with androgenic hormone production. The androgenic gland cells of Macrobrachium idella possess vacuolated cytoplasm and pycnotic nuclei during winter in Calicut; this is the time when physiology of the shrimp is tilted in favour of somatic growth (Sreekumar et al., 1980). This observation seems to suggest that hormones favouring somatic growth (MIH-MH) may suppress androgenic gland activity in adult males.

In isopods, the function of the androgenic glands is controlled by a protocerebral inhibitory centre; removal of this centre results in hyperplasia and hypertrophy of the androgenic gland cells (Legrand and Juchault, 1970). Conversely, in Orchestia gammarella, a cervical factor of the male brain which is necessary for the development of the testicular germinative zone, is itself under the control of androgenic hormone (Berreur-Bonnenfant, 1967).

Hetero- and homoplastic transplantations of gonads without or with androgenic glands in Lysmata seticaudata, Leander serratus and Cranger cranger show that a hormone, other than the androgenic hormone or neurohormone of eyestalks, is necessary for the maintenance of the germinal cells and of spermatogenesis (Touir, 1977b).

1.9.3. Reproductive Metabolism

The hepatopancreas serves as a centre of storage of organic reserves in crustaceans. The reserves are mobilised cyclically to meet the demands of reproductive and somatic growth under the influence of hormones (see K.G. Adiyodi and R.G. Adiyodi, 1970; Anilkumar, 1980; R.G. Adiyodi, 1985; R.G. Adiyodi and K.G. Adiyodi, 1970, 1972; R.G. Adiyodi and Subramoniam, 1983). In female Brachyura, a distinct antagonism exists between somatic growth and reproductive growth (R.G. Adiyodi and Subramoniam, 1983) and the physiological tilt prevalent at the time decides the nature of response of the animal to eyestalk ablation (Kurup and R.G. Adiyodi, 1981). Studies in our laboratory on Paratelphusa hydrodromous have shown that the intermoult of adult male crabs is divisible, like that of adult female crabs, into a somatic and a reproductive phase; in males during the somatic phase the physiology is tilted to favour somatic and

regenerative growth and during the reproductive phase to favour spermatogenesis and semen production (Mathad, 1983; Kurup, 1983; K.G. Adiyodi and R.G. Adiyodi, 1976; Suma, unpublished). Anilkumar (1980) has suggested that hepatopancreatic lipid, protein, oligosaccharide and polysaccharide may be utilized for yolk synthesis in Paratelphusa hydrodromous. A possible correlation between metabolic demands of ovary and taurine level of hepatopancreas of Carcinus maenas has been reported recently by Pochon-Masson et al. (1984). Though hepatopancreas is known to play a key role in reproduction in female crustaceans, next to nothing is known of the role of hepatopancreas in reproduction in male crustaceans. This prompted us to study the biochemical composition of the hepatopancreas of O. platytarsis and our findings are summarized in Chapter 4.

1.10. CONCLUDING REMARKS

From the foregoing review, it is obvious that our knowledge of the reproductive physiology of male decapods (and of male crustaceans in general) is far from satisfactory. Studies were, therefore, initiated in our laboratory to fill some of these gaps in different decapods. As a part of this programme, Mathad (1983) investigated the biochemistry and physiology of semen in two freshwater crabs, Paratelphusa

hydrodromous and Barytelphusa cunicularis; Muthuraman (1985) studied the male reproductive physiology of the marine Indian prawn, Penaeus indicus; Sreekumar (1985) of the freshwater shrimp, Macrobrachium idella; Hebbar (1982) of the freshwater crab Paratelphusa pulvinata, and Varghese (1984) of the freshwater crab Gecarcinus stenlops. Krishnakumar (1985) investigated the physiology of storage of semen in spermatheca of some female decapods. We have chosen Ocypode platytarsis for investigation because male reproductive physiology of marine crabs has been so far only poorly investigated in our laboratory; furthermore, we could benefit from comparisons of our results with those of freshwater species investigated by our colleagues and also understand how male reproductive physiology is altered by two types of habitat.

The main emphasis of this work is on histological and physiological aspects of semen production, chemical composition of the seminal plasma, processes of spermatophore formation, seasonal variations in various chemical constituents of the male reproductive system and endocrine influences in male reproductive physiology. The coral-shaped accessory sex gland of this species, which can be classified as a true collateral gland, was first reported by us (Sukumaran and K.G. Adiyodi, 1980)

and a detailed account of its morphology, physiology and biochemistry can be found in various chapters of this thesis. A number of gaps in our knowledge of male reproductive physiology have been filled and several areas of work suggested for future investigations. Some aspects of this work were presented as abstracts to national and international symposia, a list of which is appended on page 341.

Chapter II

Biology of the ghost crab, *Ocypode Platytarsis* Milne Edwards

M Sukumaran “Certain aspects of reproductive biology of the ghost crab, *Ocypode Platyt Aris* with special reference to the male” Thesis. Department of Zoology, University of Calicut, 1985

2.1. INTRODUCTION

A rich fauna flourish along the coasts of tropical countries. The brachyurans are a prominent group of invertebrates that inhabit the sandy beaches. Breeding and moulting seasons of a species of crab could vary in different geographical areas of its distribution. This is because reproductive activity and moulting are closely linked to several extrinsic factors such as temperature, salinity, tidal variations, etc. In temperate countries, the breeding season of many invertebrates is programmed to coincide with season or seasons of increased plant production. To many marine animals, the intertidal zone is a suitable habitat, inasmuch as it provides rich nutritional resources in the form of vegetation, organic debris and tidally deposited organic substances rich in microflora and stranded micro-organisms. Tide is thus a decisive factor in determining behavioural and ecological characters of intertidal fauna (Bliss, 1983 for review).

Observations on ocypodid crabs in benthic zone have revealed that feeding and height of the habitat are two of the major factors connecting crabs with the substratum of habitat (Ono, 1965). In ghost crabs, Ocypode ceratophthalmus and O. kuhlii, the feeding behaviour and food vary in some respects due to difference in habitat, but both species have similar types of

mouthparts (Jones, 1972). Some species of Uca inhabiting the intertidal zone exhibit activity rhythm synchronous to tide (cf. Kaestner, 1970). The distribution of Arrhis phyllonyx (Amphipoda) is influenced by temperature (Sainte-Marie and Brunel, 1983).

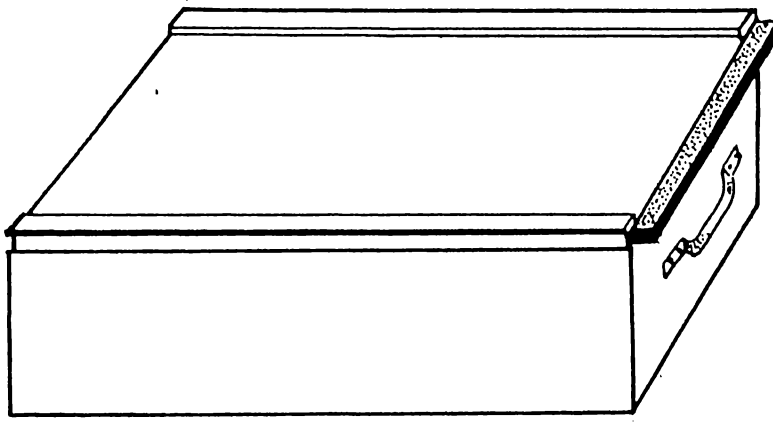
Aspects of biology of the ghost crab Ocypode platytarsis M. Edwards, such as habit, habitat, food and feeding behaviour, and breeding and life cycles were the first to be investigated by us, as such information is necessary to make a meaningful analysis of the reproductive activity of this species.

2.2. MATERIALS AND METHODS

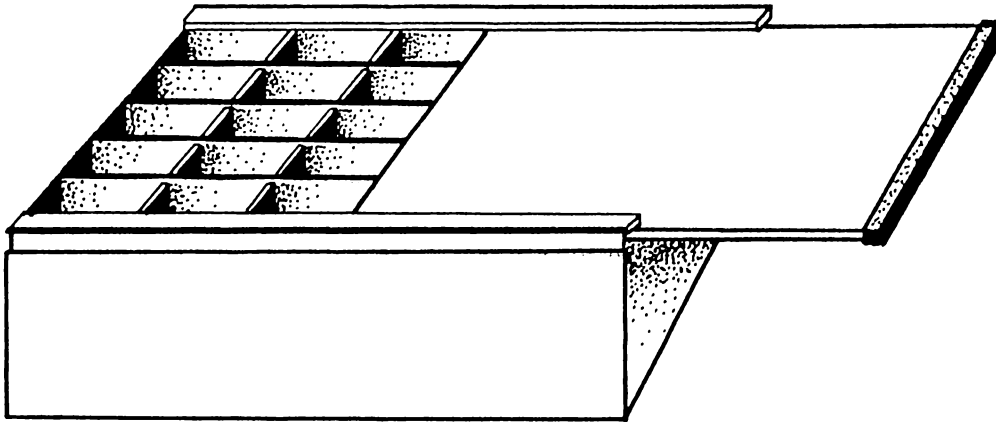
2.2.1. Method of Collection

Ocypode platytarsis, used in this study, were collected from the sandy beaches of Calicut (11.15°N , 75.49°E). Sexually mature crabs being nocturnal, collections were made chiefly during night between 19.00 and 23.00 hours. Specimens were rather rare on the shore during full-moon days, as they confined themselves either to the burrows or to very near the burrows. Although O. platytarsis were available on the shore almost round the year, catches were maximal in summer (February-May) on new moon days. From June to August (the monsoon season) the crabs were not so abundant.

- Fig. 2.1 A. A dorsolateral view of the collection box (diagrammatic)
- B. The collection box is opened to show cubicles (see text for details)



A



B

Fig 2.1

Fig. 2.2 Ringed net used for making collections
of O. platytarsis

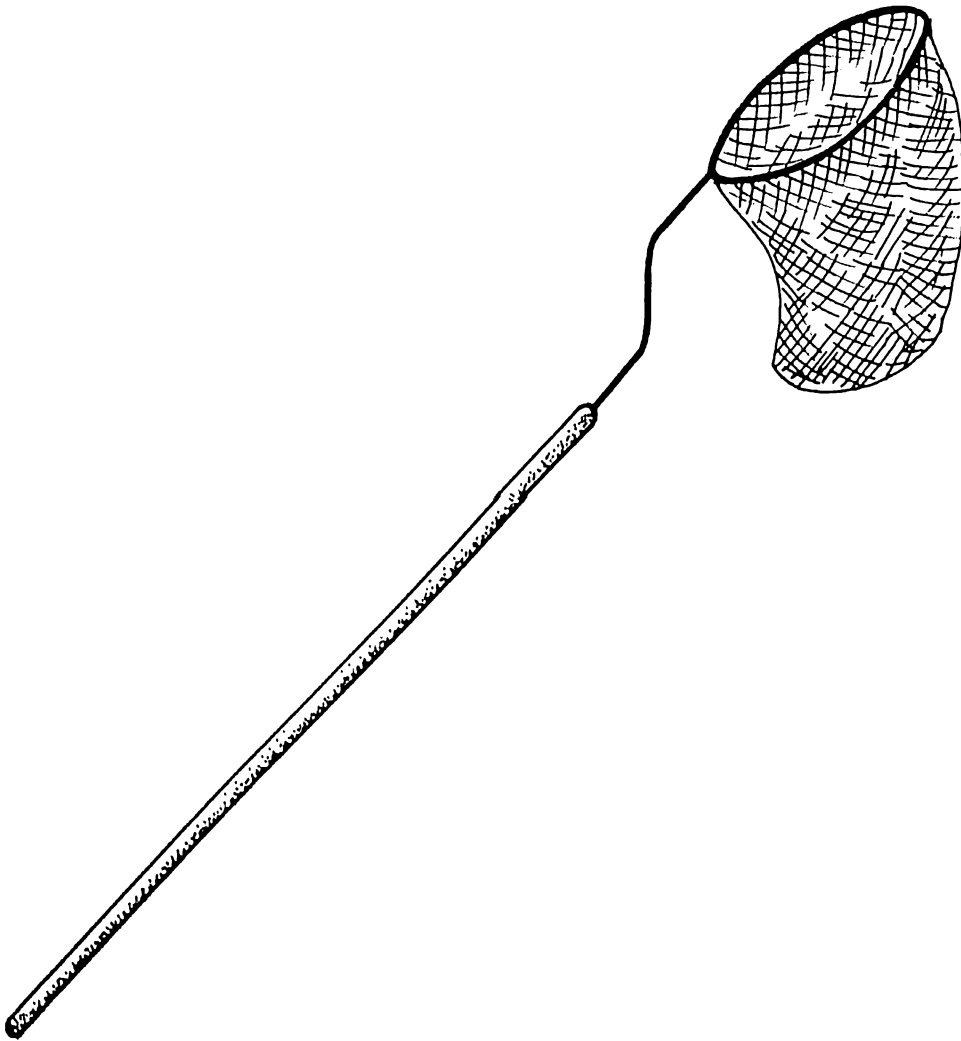


Fig 2.2

O. platytarsis exhibited variations in activity in relation to tidal pattern, lunar periodicity, etc. At the flash of torch-light, the animal was not arrested; instead, it showed a tendency to flee either to the sea or to the safety of its burrow. O. platytarsis preferred more often to escape into breakers rather than break into the burrow of a stranger. Only when hard pressed did they enter burrows of other crabs, provided they were large enough to accommodate them.

On moon-lit nights, the crabs (large ones) which were generally confined to their burrows, could be 'picked up' by a gloved hand after digging the burrow peripherally. As the juveniles (<2.5 cm carapace width) occur largely in the vicinity of their burrows and escape into the burrows as one approaches them, they could be collected only by digging the burrows.

During the new-moon quarter of the lunar period when the sandy shore seems to reach generally down to the breakers, O. platytarsis could be seen freely fending for themselves in shallow water as well as on shore above the water line. Specimens bathed by waves were easily collected using an ordinary nylon net. Those running to the shore were caught using a ringed net (Fig.2.2), comparable to the one generally used to catch butterflies. The ringed net was made of an iron-ring (60 cm in diameter)

to which was fitted a loose net bag. The iron-ring was invariably thin and therefore the net was light to carry. The net was made of ordinary fishing net or other strong nylon material and was attached firmly to the rim of the iron-ring so that the central part was loose and free. A thin strong wooden handle about 1.75 metre long, was attached to the iron-handle of the ring on one side. The specimens were trapped into the net bag by placing the ring flat on the sand over the running crab. The crabs were thus captured without any injury.

2.2.2. Mode of Transport

Specimens of O. platytarsis cannot be transported packed in small containers as they are vigorous and active and many of them lose their limbs in transit due to squabbling. Aerated individual polythene bags were of not much help either since large O. platytarsis could easily tear out of the bag with their powerful chelae. Wooden containers with isolated chambers (cubicles) were therefore specially designed to transport specimens from the shore to the laboratory (about 25 KM away). The box (43 cm x 43 cm x 20 cm), made of plywood, was divided into cubicles (size 7 cm x 7 cm) with thin plywood partitions so that at least 25 cubicles could be lodged within one box (Fig. 2.1). A sliding lid,

attached to the broad (upper) side of the box, made it convenient to transfer crabs to the cubicles. A handle was fixed on to the narrow (left/right) side of the box along which the lid was pulled out; hence the lid did not slide-open, when the box was lifted up. Some holes were made at the bottom of the box to permit air circulation to each cubicle. With this device, it was possible to ensure the safety and comfort of each crab during transit and to reduce mortality or injury during transit to zero.

2.2.3. Laboratory Culture

In the laboratory, the crabs were reared in small groups (2-3 numbers) in specially constructed cement tanks (60 cm x 60 cm x 30 cm) laid with sea water and wet beach sand. Special care was taken to clean the tanks at regular intervals. The sand laid at the bottom of the tanks was frequently wetted with fresh sea water to ensure near natural conditions. In laboratory cultures, the rate of mortality was relatively more in winter. The cultured crabs were fed ad lib. almost daily with fresh, sliced ox liver.

2.3. OBSERVATIONS

2.3.1. Animal

Adult O. platytarsis (Fig. 2.3) have a yellowish



A



B

Fig. 2.3

carapace, whereas the ventral side is straw white in colour. The colour of carapace shows slight variation in relation to the moulting cycle. For example, intermoult crabs have a golden yellow colour, while postmoult or premoult crabs are pale-yellowish in colour. Moreover, the carapace of premoult crabs usually appears somewhat puffy. Fully grown male O. platytarsis have a maximum carapace width (cw) of 6 cm, and females 5.6 cm only. Sexual dimorphism is distinct in O. platytarsis as in many other crabs: the males have a narrower abdomen and larger chelae than females. Sex differentiation becomes apparent right from the time the juvenile crabs have attained a cw of about 1.5 cm. In O. platytarsis, the chelae are asymmetrical; usually the right one is larger in size. Propodus of the chela is white, while the dorsal region (corpus and merus) is yellowish in colour. The cylindrical stalked eyes are provided with "supra-optical horns" (eye-stylets). The yellowish eyestalks are held vertically as the animal moves.

2.3.2. Habits

O. platytarsis is agile and very active. Ocypodids are sometimes treated as land crabs but are genuinely amphibious. Adult O. platytarsis is nocturnal, getting active by dusk. During the day they hide in burrows, the mouth of which is plugged with sand. However,

juveniles can be seen moving along the shore even during the day. During the full-moon quarter nights, O. platytarsis are rare on the shore. When tide is low, as during new-moon quarter nights, large numbers of them (mostly females) wander along the shore just above the water line. Good runners though, O. platytarsis are only poor swimmers. The crabs flee to water or to their burrows when they come across any moving objects within 8-12 metres. During evenings in summer months (18.30-19.00 hrs), the crabs (both males and females) come out of their burrows in large numbers and plunge themselves into the waters. As the breakers wash the shore, the crabs adhere to the sand to prevent being completely washed off, although they are moved to some distance by the force of the wave. Casual observations during morning hours have revealed that O. platytarsis hide in burrows long before dawn.

The male crabs excavate their burrows generally between 19.30-20.00 hrs, when normal feeding activity has been completed. This does not mean that they do not feed after the peak feeding period. Food particles in the vicinity of the burrows are eaten even during the late hours. During burrowing, the crab bring out each time fresh moist sand from the bottom which it holds within the chelae and the legs and spreads on one side

Fig. 2.4 Burrow of O. platytarsis (occupied by an adult male crab)

Fig. 2.5 Juvenile O. platytarsis

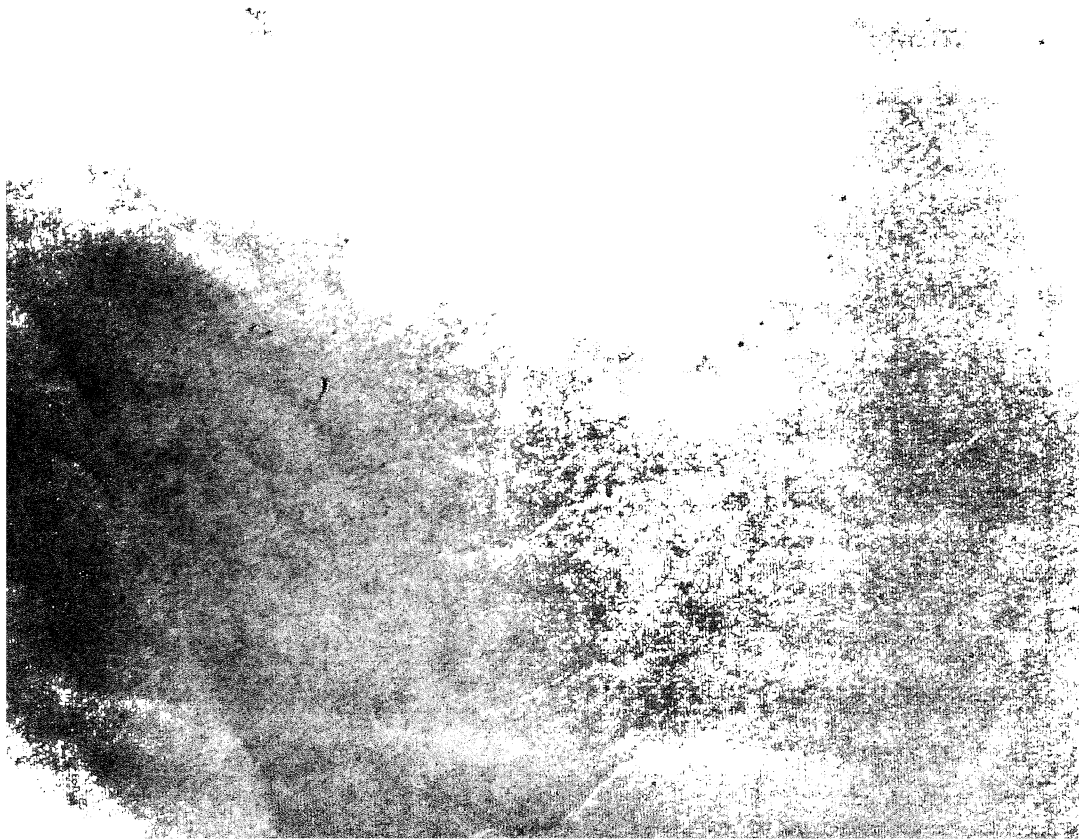




Fig. 2.4



Fig. 2.5

of the burrow. Such loose, newly dug sand near the mouth of a burrow usually betrays the presence of crab in the burrow. Generally, burrows (Fig. 2.4) are straight or slightly tortuous. O. platytarsis dig their burrows by lateral movement of the body.

A female and male are frequently seen together in one burrow. Male crabs may occupy solely some burrows, but only rarely have we observed a female occupying a burrow all by herself.

In aggressive defence behaviour, the chelae are raised above the level of the body. The larger chela is the one usually used for defence. It also ejects out two jets of watery secretion from the upper part of the mouth and produces a 'cracking sound' similar to cricket's call.

2.3.3. Food and Feeding Pattern

O. platytarsis are voracious feeders and scavengers. They feed mainly on dead animals (fishes, snakes, etc.) which are washed ashore. In polluted beaches, night soil and other decomposing organic matter also form their food, but we have never seen them feeding on plant matter. Frequently, adult O. platytarsis feed on their own juveniles and on crabs belonging to other species. In the laboratory, they voraciously devour fresh ox liver.

PROFILE OF THE SANDY BEACHES OF CALICUT
DURING TWO DIFFERENT PHASES OF LUNAR CYCLE

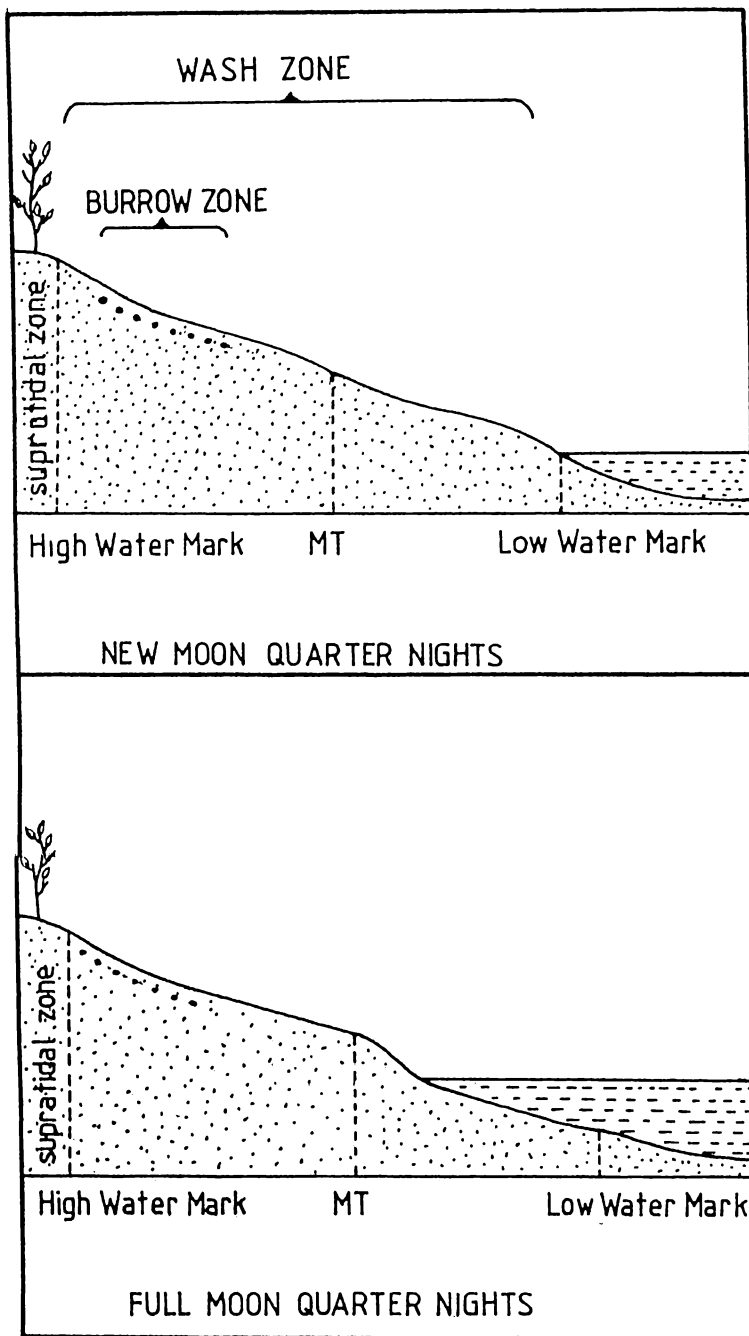


Fig. 2.6

Depending on the size of the individual, daily intake is 2.0-2.5 gm of liver per day in the laboratory. The crabs detest ground-nut cakes or vegetables given as food. O. platytarsis carry food to a comfortable place, if the piece of food is of convenient size holding it with the larger chela; using the smaller chela, the food is next torn off into smaller pieces and each piece pushed into the mouth. The powerful mandibles of this species can crack even the chitinous limbs or chelae of other crabs.

2.3.4. Habitat

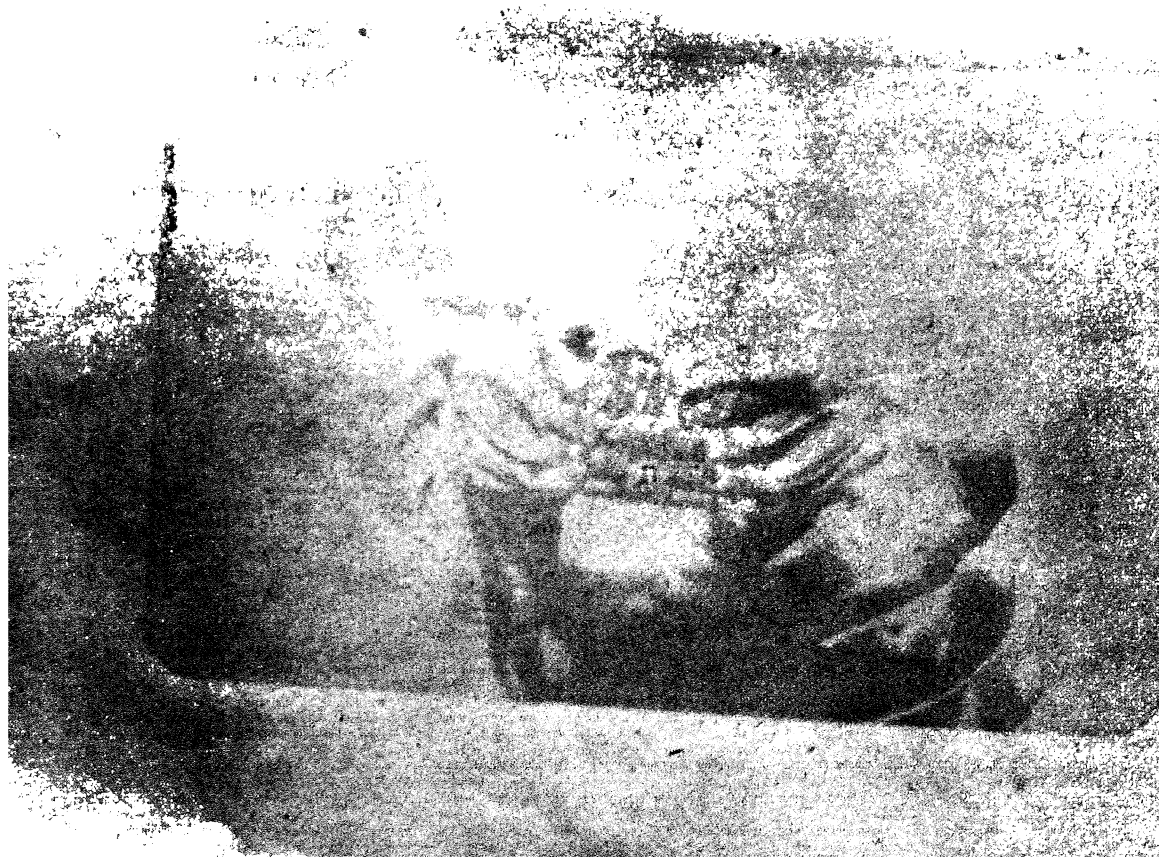
The sandy shores of Calicut are poor in vegetation. Another species of ghost crab, Ocypode ceratophthalmus (Pallas) (Fig. 2.7) is sympatric with O. platytarsis in Calicut. cursory resemblance notwithstanding, the two species can be easily distinguished: the adults of O. ceratophthalmus are smaller (cw 4.5 cm) than the adults of O. platytarsis. O. ceratophthalmus have a brownish shading on their carapace, and two large, blackish "inverted-coma shaped" markings toward the posterior part of the carapace. Dark-brown patches are seen on the ventral side of the body, the legs and abdomen. 'Hairs' thickly cover the legs of O. ceratophthalmus; such hairs are absent in O. platytarsis. There are also



Fig. 2.7 Adult male O. ceratophthalmus

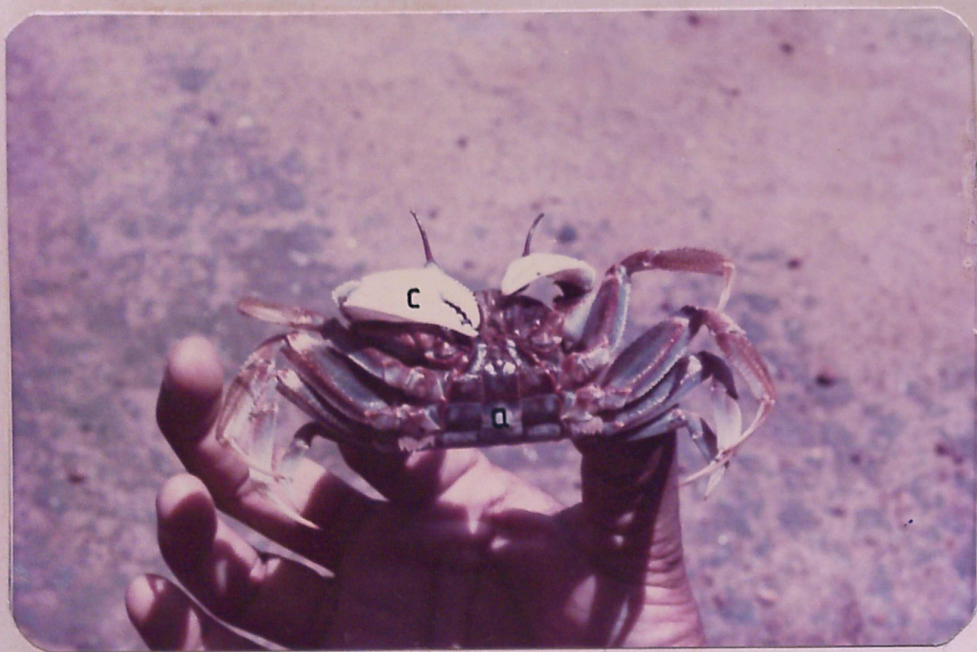
A. dorsal view; B. ventral view

a - abdomen; c - chela





A



B

Fig. 2.7

differences between the two species in their habitats. The burrows of O. platytarsis are usually located a few meters away from the water-line, while those of O. ceratophthalmus are much closer to the water-line. Burrows of O. ceratophthalmus are not as deep as those of O. platytarsis. O. platytarsis are more vigorous and active than O. ceratophthalmus. O. ceratophthalmus prefer areas where O. platytarsis are rare. Adult ovigerous females of O. ceratophthalmus are frequently seen lying in loose sand in the upper reaches of the beach, with their eyes alone projecting out (body buried) of the sand. O. platytarsis do not exhibit this behaviour pattern.

In relation to the tidal pattern, variations in beach terrain appear to exert some influence on behaviour of the crabs. Thus, for a few nights preceding and succeeding the full-moon days, when a terrace-like formation (Fig. 2.6) is present on the beach, except for the male crabs in their burrows, not many individuals are seen on the raised platform; most of the crabs are seen further down, very near the breakers. During these days, the number of crabs that wander out is relatively low. During new-moon quarter nights, when the tide is low and terrace-formation is absent, large number of O. platytarsis (majority females) are seen foraging just

above the water level. In these nights, many males are seen in burrows.

Larger crabs generally make deeper burrows; the diameter of the burrow also depends upon the size of the individual. Smaller crabs make burrows near the water-line where sand particles are smaller and richer in sediments. Earlier studies by Pillai (1982) from the same locality have revealed that there is no significant difference in water temperature (about 32°C) during day and night in April/May. However, the surface temperature near the burrow during day time is $45.7 \pm 0.5^{\circ}\text{C}$ and at night $32.4 \pm 1.4^{\circ}\text{C}$. In the burrows (10 cm below), during day time, the temperature is $36.1 \pm 1.1^{\circ}\text{C}$ and at night $35.7 \pm 1.6^{\circ}\text{C}$. The percentage of soil moisture in burrows is 22.45 ± 1.9 (Pillai, 1982).

2.3.5. Breeding and Life Cycle

O. platytarsis are continuous breeders: ovigerous females have been found along the shore almost round the year. However, there appear to be two peak spawning seasons, one during April/May and the other during September/October. Berried females appear to segregate themselves from the general population. The eggs of O. platytarsis, deep orange in colour, have a diameter of 0.4 to 0.5 mm and the clutch size ranges between

1,500 and 2,000. However, with advancing embryogenesis, the colour of eggs changes to a dark brown. Field observations reveal that 60-70% of the male crabs collected during October/November are in premoult.

Juveniles of O. platytarsis (Fig. 2.5) are seen in large numbers during June-July and November-December. The carapace of young crabs (cw < 3.2 mm) is dull green and is ornamented with a white network-like design. The ventral side is pale white. In O. ceratophthalmus, the characteristic brownish patches are apparent in juveniles (> 1 cm cw) themselves. On the contrary, in O. platytarsis, the colour of the carapace shows a gradual change with maturation. In young O. platytarsis of cw 3.3-3.9 cm, the carapace is yellowish and has a white network-like pattern; the carapace acquires the typical adult coluration only as crabs attain a cw of 4 cm.

2.4. DISCUSSION

Ocypode platytarsis is far more active than other species of crabs studied in our laboratory, viz., Paratelphusa hydrodromous, Paratelphusa pulvinata, Barytelphusa cunicularis, Ocypode ceratophthalmus and Gecarcinucus steniops. Although relatively small in size compared to Scylla serrata and Portunus pelagicus,

O. platytarsis is larger than most of the previously mentioned species. Asymmetry of the chelae found in O. platytarsis is also observed in some other species such as P. hydrodromous and O. ceratophthalmus. The period of activity of O. platytarsis varies with tidal and lunar cyclicity. Adult males of the shore crab, Hemigrapsus oregonensis show tidal and diurnal rhythm; the greatest activity observed was during the dark high tide (Batie, 1983). Environmental factors such as the intensity of illumination (even the presence of cloud), temperature of air, relative humidity of air, availability of protective cover for quick retreat, etc., influence the daily activity of Gecarcinus lateralis (Bliss et al., 1978).

On full moon days, O. platytarsis are very scarce on the shore. Paulraj (1980) reported a nyctymeral and lunar variation in autotomy response of this species. On new-moon days, autotomy frequency had a 'sinusoidal' pattern. On full-moon days and last quarter days, there was an asymmetrical variation. Moreover, at high tides during night, autotomy response was very low in O. platytarsis (Paulraj, 1980). Changes in behaviour in relation to tide and lunar periodicity is not unique to O. platytarsis. In many intertidal organisms, physical factors may inhibit or promote the reproductive success

of the individual. For instance, the stomadopods Gonodactylus zaca and G. falacatus oviposit during neaptides or transition from spring to neaptides (Reaka, 1976). A sand isopod, Exocirolana chiltoni shows daily and monthly activity rhythms in relation to tidal agitation (Enright, 1972).

Our observations on feeding behaviour of O. platytarsis show that the species is basically carnivorous, but the food is diverse and includes carrion. Under laboratory conditions, the crabs which were fed on ox liver appeared healthier than those fed on other food-stuffs. In the field, O. platytarsis are both scavengers and predators. Cannibalism is also common in this species as in Paratelphusa hydrodromous. The land crab, Gecarcinus is primarily herbivorous, although lizard, members of its own species, eggs of birds, feather and bone also form part of its food (Bliss et al., 1978). A shift from herbivorism to carnivorism or to mixed diet is not uncommon in some crabs during postembryonic development and growth (e.g., P. hydrodromous, Gecarcinucus steniope). Ono (1965) reported that in some species of oecypodid crabs, nearly all nutrients assimilated in a day are metabolized the same day.

It is possible that sand piles at the mouth of the burrow of male O. platytarsis may serve to attract the

females. Male Ocypode seratan, for instance, erect sand pyramid of about 16 cm height, about 40 cm away from the mating burrow. The pyramid and burrow are connected by a tramped path. The females, attracted by the pyramids follow the path into the burrow which is occupied by the male. The male produces vibration signals when the female gets nearer to the burrow. The pyramid also acts as a stimulus to mature males for territorial defence. The male stays in the mating burrow for 4-8 days, even foregoing food. After mating period (April/May) the male digs simple resting burrows (cf. Kaestner, 1970). Earlier investigations by Paulraj (1980) have shown that O. platytarsis construct three types of burrows. Maturing males and females construct 'U'-type or 'Y'-shaped burrows, whereas mature males make spiral or straight 'copulating burrows' (Paulraj, 1980).

It is not known if the 'cracking-sound' produced by O. platytarsis has any acoustical significance. The acoustic signals produced by males from different species differ from one another, permitting females to distinguish calls of males belonging to the same species from those of heterospecific males (Salmon and Hyatt, 1983). Social signals in crustaceans appear to be derived independently even within closely related species (Bliss, 1983). Calling sounds of O. ceratophthalmus and O. laevis are produced by

rapping or stridulation (Bliss, 1983).

The threat posture (behaviour) of O. platytarsis is of interest to ethologists, but is not unique to this species among crustaceans. When threatened, grapsoid crabs, for example, put up a characteristic display, referred to as 'high intensity lateral merus', in which the chelae are forcibly drawn out to either side (Wright, 1968 MS). The threat behaviour of O. platytarsis is comparable to that of Uca vocans (cf. Kaestner, 1970). When irritated, O. platytarsis produce a jet of watery secretion aimed probably at the intruder; the pistol shrimp Alpheus also produces a jet of water when forced to defend the territory, or when confused with other males and even in the course of catching the prey (cf. Kaestner, 1970).

In adult O. platytarsis, the optimum temperature for activity is around 32°C. In the land crab Gecarcinus lateralis, the favourable temperature for maximum activity in summer is below 30°C. In G. lateralis, low temperature adversely affects the activity of the crab, whereas favourable temperature and abundant moisture lead to the onset of activity (Bliss et al., 1978).

Our studies have shown that two species of ocypodid crabs (O. platytarsis and O. ceratophthalmus) are

sympatrically distributed along the Calicut shore. This is not unusual among crustaceans. Ecological studies on isopods have shown that very closely related species may occur together in an apparently similar habitat (Naylor, 1972). According to Ono (1965), among ocypodid crabs, those holding territory of fixed size live in higher regions of tidal flats, whereas those having territory of changing size live in plains. The crabs which hold fixed territory understandably show severe fighting behaviour. Chlorinity is considered to be an important factor in determining the distribution of these crabs (Ono, 1965).

Mechanisms preventing interbreeding between O. platytarsis and O. ceratophthalmus, which overlap in distribution, could be diverse. Anatomy of the reproductive system of O. platytarsis differs from that of O. ceratophthalmus: for instance, the mid vas deferens of O. ceratophthalmus has many protuberances and is sac like (unpublished data), while mid vas deferens (MVD-I) of O. platytarsis has uniform girth and is coiled (see Chapter III for details). In female O. platytarsis, it is reported (Pillai, 1982) that the size of individual oocytes increases appreciably in the course of vitellogenesis whereas in O. ceratophthalmus, the oocytes do not increase in size appreciably during the vitellogenic cycle

(Krishnakumar et al., 1979). Furthermore, differences between the two species in external morphology such as size and colouration and in ecological distribution (not well marked) could promote recognition of conspecific individuals and act as a deterrent to interspecific breeding. As reproductive cycles of O. ceratophthalmus along the Calicut coast has not yet been investigated, we do not know if breeding cycles of the two species are programmed differently as in some sympatrically distributed hermit crabs. Reese (1968) found that peak breeding periods of two species of hermit crabs occurring in the same habitat do not coincide with each other and thus they avoid the possible competition for shell selection. The sand crabs, Hippa strigillata and H. pacifica are similar in appearance and are found in the same intertidal zone, but interbreeding is prevented as their life cycles vary (Efford, 1972).

Different species of the same genus may show variations in programming their breeding cycle in response to variations in environment. For instance, all species of Emerita in temperate seas are seasonal breeders (breeding cycle centred around summer months), while those in tropical shores breed continuously throughout the year. It is suggested that in such cases breeding activity follows the classical temperature-dependent pattern. E. asiatica,

inhabiting tropical beaches, is a continuous breeder (Subramoniam, 1977). But Albunea symnista, another tropical anomuran inhabiting the sandy beaches of tropics, breeds irregularly (T. Subramoniam, unpublished). Increasing day length is a stimulus for copulation in Ocypode quadrata in which the peak breeding season is during summer (Haley, 1972). Even the so-called continuous breeders may show one or more peak breeding seasons a year. The present study indicates that O. platytarsis, though a continuous breeder, has two such peak spawning seasons. The penaeid prawn Parapenaeopsis hardwickii breeds throughout the year, but the peak breeding and spawning activities occur during January-May (Nagabhushanam and Kulkarni, 1982). E. asiatica has no specific breeding season, but egg development and hatching do not occur during May-August (Subramoniam, 1977). However, the possibility of the occurrence of any factor restraining breeding activity in a marine environment is limited, as food and other ecological factors do not show much annual variation particularly in the tropics. On the contrary, the situation in estuary is different. The hermit crab, Calibanarius longitarsus does not breed during monsoon. The major limiting factors during monsoon are the low salinity and sparse distribution of planktonic food; low salinity

leads to a high mortality of the larvae (Ajmal Khan and Natarajan, 1979a,b).

2.5. SUMMARY

1. Ocypode platytarsis, collected for our studies from the sandy beaches of Calicut, is a species inhabiting the intertidal zone of tropical sea. The burrows of adult O. platytarsis are located a few meters away from the water-line. O. platytarsis shows sexual dimorphism; sex differentiation becomes apparent right from the time juvenile crabs have attained a carapace width (cw) of about 1.5 cm.
2. Adult O. platytarsis are agile, amphibious and nocturnal, showing aggressive defence behaviour. The period of activity of O. platytarsis varies with lunar and tidal cyclicity.
3. The food of O. platytarsis is diverse. They are basically carnivorous. In the field, they are scavengers and predators. Cannibalism is also common.
4. The optimum temperature for activity of adults is about 32°C.
5. Ocypode platytarsis (Milne Edwards) is sympatric in

distribution with Ocypode ceratophthalmus (Pallas) in Calicut beach. There are striking differences in morphology, habit and anatomy of the reproductive system between O. platytarsis and O. ceratophthalmus.

6. Ovigerous females of O. platytarsis have been found along Calicut beach almost round the year. Colour of eggs changes from deep orange to dark brown with the advancing embryogenesis. Juveniles make burrows near the water-line where sand particles are smaller and richer in sediments. The carapace of juveniles is deep green with a white network-like design; the carapace acquires the typical adult coloration (yellow) as the young crabs attain a cw of about 4 cm.
7. O. platytarsis is a continuous breeder with two peak spawning seasons.

Chapter III

Certain aspects of semenogenesis in *Ocypode Platytarsis* Milne Edwards

M Sukumaran “Certain aspects of reproductive biology of the ghost crab, *Ocypode Platyt Aris* with special reference to the male” Thesis. Department of Zoology, University of Calicut, 1985

3.1. INTRODUCTION

Knowledge on reproductive biology of male crustaceans is uneven. Role of androgenic hormone on primary, secondary and behavioural sex characters of the male has been extensively studied, largely by Charniaux-Cotton and her collaborators (Charniaux-Cotton et al., 1985); a wealth of information is also available on structural details of crustacean spermatozoa and on the process of crustacean spermatogenesis (Pochon-Masson, 1983 for review). However, apart from the contributions of Barnes (Barnes, 1962a,b, 1963; Barnes and Finlayson, 1962) on cirripedes and Sabeeda et al. (1974) and Mathad (1983) on Paratelpusa, very little is known on the composition of semen and on the role of various accessory glandular elements in the production of the spermatophore and seminal plasma in the Crustacea. The present investigations on the ghost crab, Ocypode platytarsis were undertaken with a view to throw some light on these aspects in the **Brachyura**.

3.2. MATERIALS AND METHODS

3.2.1. Animals

Sexually mature specimens of the ghost crab, Ocypode platytarsis having a carapace width of 5.0 to 5.5 cm were

collected from Calicut beach during May-June. The method of collection, transport, etc. are as described in Section 2.2.

Moulting stages of male O. platytarsis were determined by observing setal formation in the maxillipede (comparable to that in pleopods, after Drach, 1939; Anilkumar, 1980). Only crabs in intermoult stage were used in the present study. Early stages of intermoult are characterised by the appearance of pigments on the blade of the appendage; the latter appears rather translucent. The setal base is more prominent and thick. By this time, the exoskeleton of the animal has hardened and the carapace has a golden yellow colour. The animal is very active. During late intermoult, the pigments appear in greater density. The setal base is very distinct. The animal continues to be very active and feeds normally. The carapace retains the golden yellow colour.

3.3.3. Tissue Analysis

The male reproductive system was carefully dissected out in normal saline. For in vivo studies, various parts of the male reproductive system were stained with methylene blue solution (0.5%) without using any fixative and squeezed gently under the coverslip prior to observation under the light microscope. Squashes of testis and contents of vas

deferens and spermatheca were examined under phasecontrast microscope.

For histological studies, the crabs were sacrificed and different regions of the male reproductive system and spermathecae were immediately fixed separately in 10% formol-saline or Bouin's fixing fluid. Paraffin sections (6.0 to 7.0 μm in thickness) were stained with haematoxylin and alcoholic eosin.

The basic biochemical nature of the different components of semen was studied by testing solubility in different solvents (see Mathad, 1983). Contents of the mid vas deferens and coral-shaped accessory sex gland were expressed out and kept for observation in basic solutions (NH_4OH , KOH - 1% and 50%), acidic solutions (HCl , HNO_3 - 1% and 50%) and salt solutions (NaCl , $(\text{NH}_4)_2\text{SO}_4$ - 1% and 50%). After keeping in these solvents for 30 minutes, the samples were observed under microscope.

3.2.3. Histochemical Analysis

For histochemical studies, the tissues were fixed in Bouin's fluid, 10% formol-saline, or Carnoy's fluid. Chilled sections, 12-13 μm thick, were used for the localization of lipids. For the various histochemical tests, we have largely followed Pearse (1968) and Chayen et al. (1973), unless otherwise stated.

3.2.3.1. Proteins. Proteins containing acidic groups were detected by the mercury bromophenol blue method. Histochemical tests were also conducted to demonstrate the presence of proteins containing basic groups (Johri and Smyth, 1956), tyrosine (Millon's reaction modified by Baker), α -amino acids or protein containing α -amino acids (Yasuma and Ichikawa, 1953) in various regions of the male reproductive system.

3.2.3.2. Carbohydrates. Periodic acid-Schiff (PAS) technique was adopted to localize 1:2 glycol groups and other PAS-positive substances. Acid mucopolysaccharides were detected by Alcian blue staining method (Steedman, 1950). Tests were also conducted to demonstrate sulphated acid mucopolysaccharides and glycogen (Best, 1906).

3.2.3.3. Lipids. For the histochemical localization of lipids, we have followed Sudan black B method (lipids in general) and Oil red O (neutral lipids) staining techniques.

3.2.3.4. Nucleoproteins. DNA was detected by Feulgen reaction (Feulgen and Rossenbeck, 1924).

In order to verify the validity of the results obtained, all observations were repeated on a minimum of five specimens.

3.3. OBSERVATIONS

3.3.1. Gross Morphology of the Male Genital System in O. platytarsis.

In O. platytarsis, each testis is located on either side of the stomach. Toward the posterior part of the gastric stomach, testes of both sides approach each other, giving a V-shaped appearance. Each testis is a flattened elongated structure richly provided with grape-like protruberences (acini) along its entire length. Each testicular half has an average length of about 25-30 mm in a crab having a carapace width of 5.0-5.5 cm. It tapers toward the antero-lateral corner of the cephalothorax at its broadest part, it measures about 2.5 mm across. The vas deferens of each side arises as a narrow duct from the posterior part of the testis. Morphologically, the sperm duct could be divided into three regions - the anterior vas deferens (AVD), mid vas deferens (MVD) and the ejaculatory duct (ED). AVD, which is much folded and coiled, leads to MVD; the latter is less folded, thicker and more elongated than AVD. The vas deferens on each side is provided with a well organized coral-shaped accessory sex gland (CSG) (Fig. 3.1) that empties its contents into the distal end of MVD. Androgenic gland is an elongated structure consisting of a number of parallel wavy strands, superficially attached to ED.

3.3.2. Spermatozoa in O. platytarsis

O. platytarsis spermatozoa (Fig. 3.2) are disc-shaped cells, 2.5-3.0 μm in diameter. In Feulgen-stained preparations, we could detect the presence of DNA in the form of a ring toward the cell periphery (Fig. 3.25). A distinct PAS-positive area, possibly representing acrosome, could be observed in the middle of the cell body. Spermatozoa showed positive reaction with mercury bromophenol blue, aqueous bromophenol blue, Millon's reagent and ninhydrin-Schiff, suggesting a high content of proteins. With toluidine blue, the spermatozoa showed α -metachromasia. Appreciable amount of lipid was also present on O. platytarsis spermatozoa (Table 3.1).

Spermatozoa exhibited the characteristic acrosomal reaction under osmotic shock, e.g., when exposed to hypotonic solutions. The reaction was marked by the protrusion of the acrosomal region thus giving the appearance of a stalked-ball. Right from testicular acini down to spermatophores distributed in the distal extremity of MVD, the spermatozoa have been found capable of undergoing eversion in O. platytarsis.

3.3.3. Histophysiology and Histochemistry of Sperm Duct in O. platytarsis: Aspects of Spermatophore Formation

Based on gross morphology, sperm duct of O. platytarsis

is divided into three regions, namely AVD, MVD and ED (Section 3.3.1), but six distinct regions can be distinguished based on histological and physiological parameters. Thus AVD could be divided into proximal AVD-I, middle AVD-II and distal AVD-III; and MVD into MVD-I and MVD-II. The sixth part is the muscular posterior vas deferens or ejaculatory duct (ED).

3.3.3.1 (a) AVD-I. In individuals having a carapace width of 5 cm, AVD-I has an approximate length of 7 mm. This is the first part of the vas deferens and in vivo observations revealed that it is completely filled with spermatozoa. Anteriormost region of the AVD-I is in the form of a very narrow duct (maximum diameter: 90 μm) bound by columnar epithelium having an approximate height of 17.5 μm ; it is wrapped by a thin layer of cells whose nature could not be identified with light microscopy. The epithelial cells which directly line the lumen are so arranged that their nuclei, instead of forming a regular row, occupy a backward position in some and a forward position in others giving a kind of zig-zag pattern (Fig. 3.3) and hence the nuclei most often give the impression of being arranged in groups of 2-8. The spermatozoa here are suspended in a secretion which showed intense positive reaction to alcian blue (pH: 2.5) and a practically negative reaction to mercury bromophenol

Fig. 3.1 Diagram showing the male reproductive system of O. platytarsis

Fig. 3.2 Spermatozoa of O. platytarsis
Formalin, Feulgen X 1200

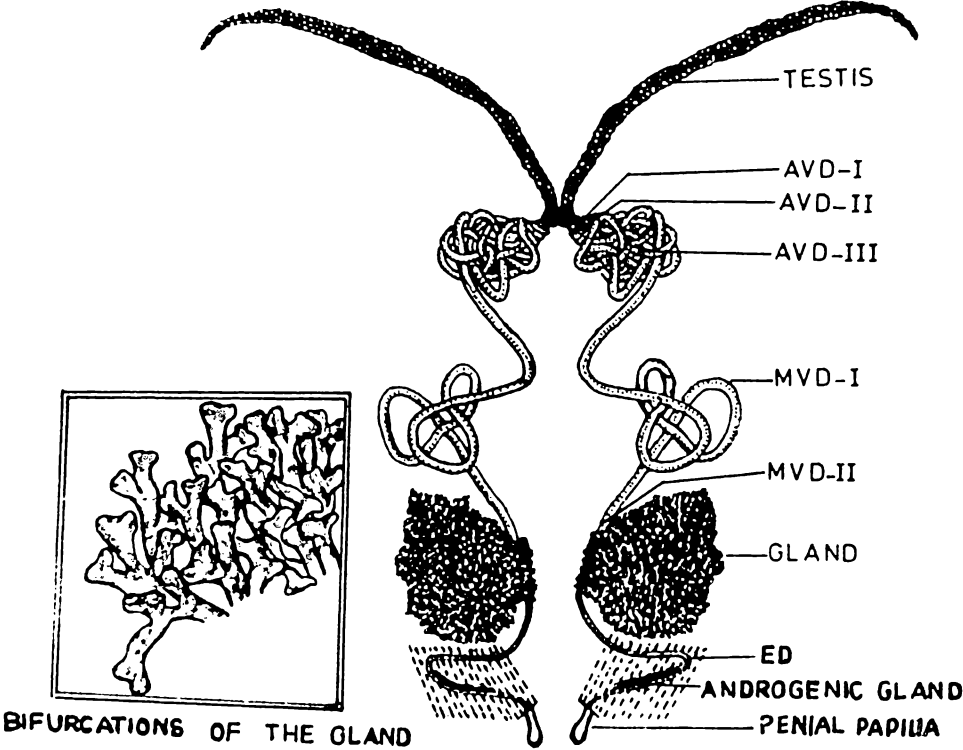
Fig. 3.3 A cross section through the anterior part of the AVD-I of O. platytarsis.

E - epithelial cells; N - nuclei;

S - spermatozoa

Formol-saline, Heidenhain's haematoxylin-
alcoholic eosin X 500

MALE REPRODUCTIVE SYSTEM IN OCYPODE PLATYTARSIS



3.1

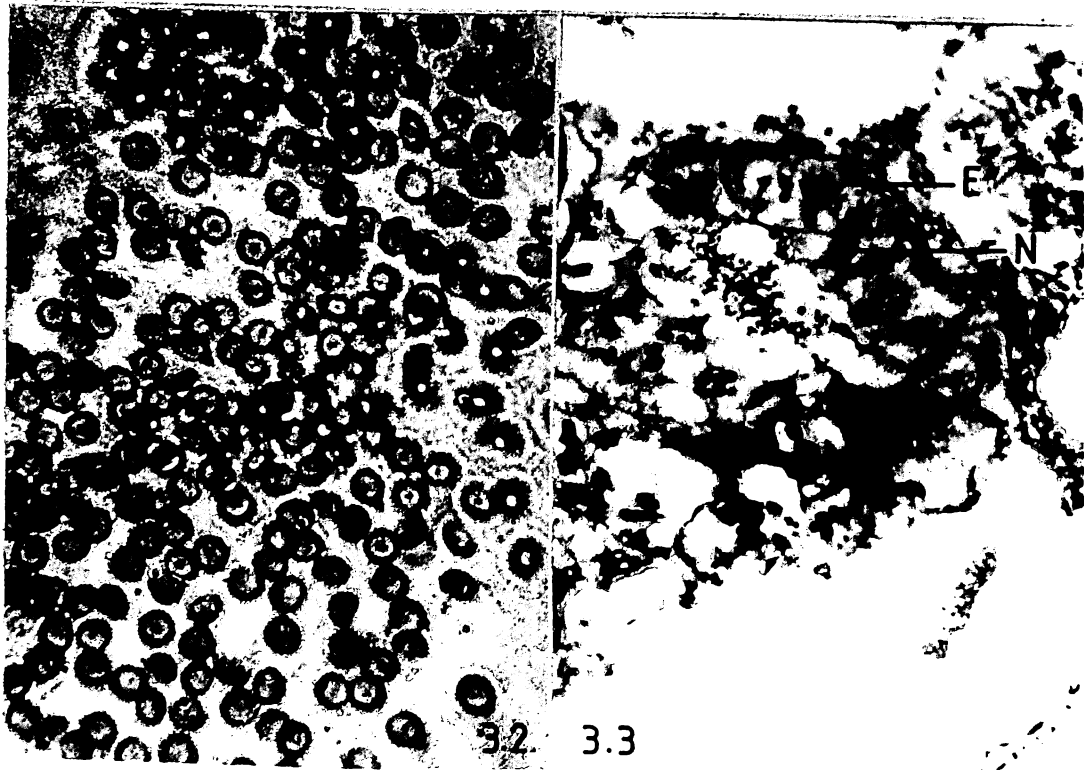


Fig. 3.4 A cross section of the posterior part of the AVD-I of O. platytarsis.

E - epithelial cells; N - nuclei;
S - spermatozoa.

Formol-saline, Heidenhain's haematoxylin-alcoholic eosin X 600

Fig. 3.5 A cross section of AVD-II of O. platytarsis showing primary spermatophores.

Formol-saline, Heidenhain's haematoxylin-alcoholic eosin X 600

Fig. 3.6 A cross section of AVD-III of O. platytarsis.
S - spermatozoa; Sp - spermatophores.

Formol-saline, Heidenhain's haematoxylin-alcoholic eosin X 200

Fig. 3.7 A squash preparation of the semen of O. platytarsis showing crystalline entities (Cr)

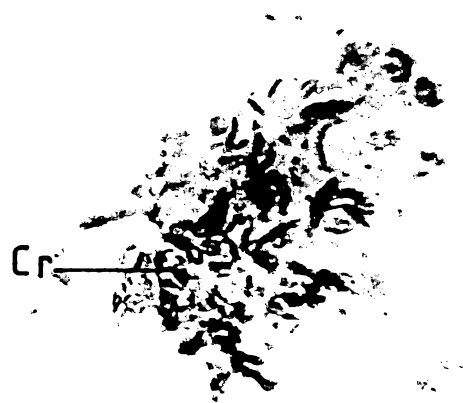
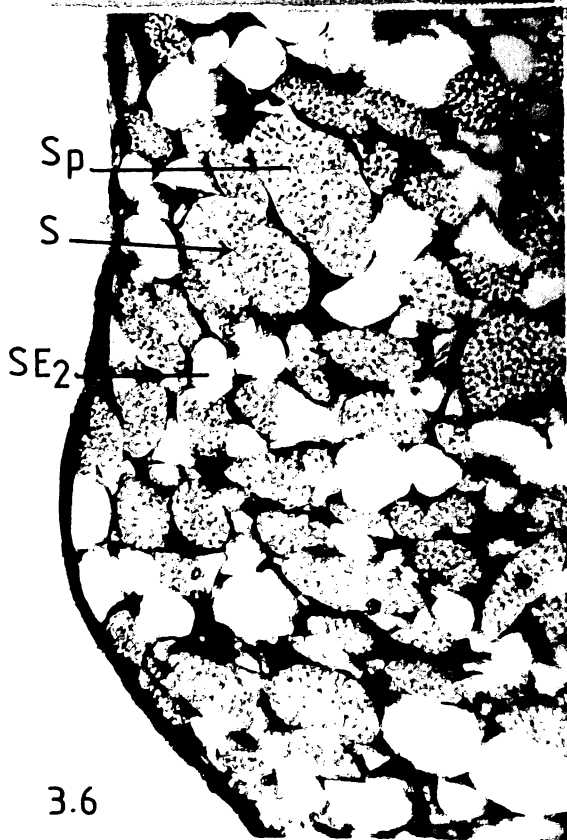
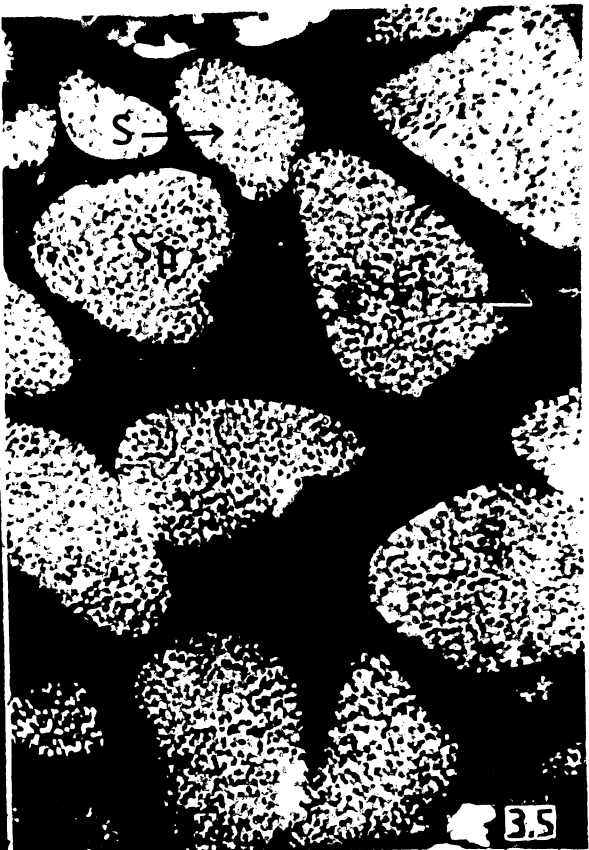
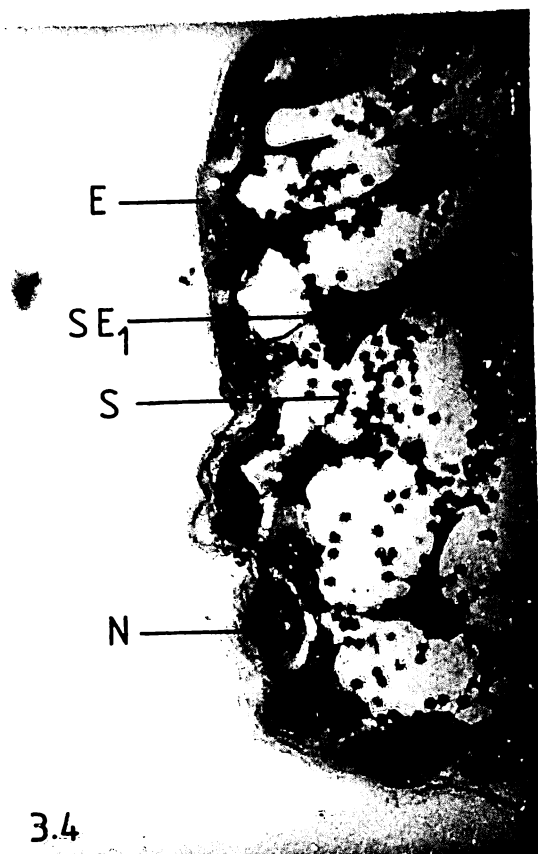


Table 3.1
Histochemical analysis of seminal components and epithelial cells of the vas deferens and CSG of
Ocypode platytarsis

Tests	Inference	Epithelium										SE-I			SE-II			
		AVD I	AVD II	AVD III	AVD I	MVD I	MVD II	ED	CSG	AVD I	AVD II	AVD-III+	MVD-I+	MVD-II+	ED	AVD-III	MVD-I+	MVD-II+
Mercury-bromophenol blue	Presence of acid groups in protein	++	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
Aqueous bromophenol blue	Presence of basic groups in protein	++	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
Millon's reaction	tyrosine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schiff only	free aldehydes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ninhydrin-Schiff	protein-bound NH ₂	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

contd.

Tests	Inference	Epithelium										SE-I		SE-II	
		AVD I	AVD II	AVD III	AVD I	MVD I	MVD II	ED	CSG	AVD I	AVD II	AVD-III+	MVD-I+	MVD-II+	ED
PAS (de Thomasi)	1:2 glycol or the equivalent amino or alkyl amino derivative or the oxidative product CHO ₂ -CO	±	±	±	±	±	±	±	±	±	±	±	±	±	±
PAS+acetylation	1:2 glycol block	±	±	±	±	±	±	±	±	±	±	±	±	±	±
PAS+acetylation+deacetylation	1:2 glycol block release	±	±	±	±	±	±	±	±	±	±	±	±	±	±
Alcian blue-PAS	Periodate-unreactive and alcianophilic (B) periodate-reactive and non-alcianophilic (R)	±	±	±	±	±	±	±	±	±	±	±	±	±	±
Alcian blue (pH:2.5)	Weakly acidic sulphated mucosubstances	B- R+	B- R-	B+ R+	B- R+	B- R+	R	R	R	R	B- R+	B- R+	R+B	R	R
Alcian blue (pH:1.0)	Sulphated mucosubstances.	±	±	±	±	±	±	±	±	±	±	±	±	±	±

. contd.

Tests	Inference	Spermatophore wall			Spermatophore ground substance	Sperms	Crystalline entity	Fibrous secreti of CSG
		AVD I	AVD-II+ AVD-III	MVD-I+ MVD-II+ ED				
Mercury-bromo-phenol blue	Presence of acidic groups in protein	-	+p	+	-	+++	+++	+++
Aqueous bromo-phenol blue	presence of basic groups in protein	-	+p	+	-	+++	+++	++
Millon's reaction	tyrosine	-	+p	+	-	+	++	+
Schiff only	free aldehydes	?	+p	+p	+	+++	-	+
Ninhydrin-Schiff	protein-bound NH ₂	?	+p	+	-	++	+	+
PAS (de Thomasi)	1:2 glycol or the equivalent amino or alkylamino derivative or the oxidative product CHO ₂ -CO	?	+	+	+	+	-	++

contd..

Tests	Inference	Spermatophore wall				Spermatophore ground substance	Sperms	Crystalline entity	Fibrous secretio of CSG
		AVD I	AVD-II+	AVD-III	MVD-I+ MVD-II+ ED				
Mercury-bromo-phenol blue	Presence of acidic groups in protein	-	+p		+	-	+++	+++	+++
Aqueous bromo-phenol blue	presence of basic groups in protein	-	+p		+	-	+++	+++	++
Millon's reaction	tyrosine	-	+p		+	-	+	++	+
Schiff only	free aldehydes	?	+p		+p	+	+++	-	+
Ninhydrin-Schiff	protein-bound NH ₂	?	+p		+	-	+	+	+
PAS (de Thomasi)	1:2 glycol or the equivalent amino or alkylamino derivative or the oxidative product CHO ₂ H-CO	?	+		+	+	+	-	++

contd..

Tests	Inference	Spermatophore wall				Spermato- phore ground substance	Sperms	Crysta- lline entity	Fibrous secretion of CSG
		AVD I	AVD-II+ AVD-III	MVD-I+ MVD-II+ ED					
PAS+acetylation	1:2 glycol block	?	+	+		±	+	-	++
PAS+acetyla- tion+deacetyla- tion	1:2 glycol block release	?	+	+		±	+	-	++
Alcian blue (pH: 2.5)	weakly acidic sulphated mucosubstances	?	++	++		+++	±	-	++
Alcian blue (pH: 1.0)	sulphated muco- substances	?	++	++		+p	±	-	++
Alcian blue- PAS	periodate-un- reactive and alcianophilic (B), periodate- reactive and non-alciano- philic (R)	?	R+B	R+B		B	R	-	R+B

contd.

Tests	Inference	Spermatophore wall				Spermatophore ground substance	Sperms	Crystalline entity	Fibro secre of CS
		AVD I	AVD-II+ AVD-III	MVD-II+ MVD-III ED	β	γp	α	β	
Toluidine blue (Kramer and Windrum)	acidic groups	-	β	β					
Bests' carmine	glycogen	-	±	±		-	+	-	+
Salivation+ Bests' carmine	glycogen block	-	-	-		-	±	-	±
Oil red O	neutral lipids	-	-	-		-	±	-	±
Sudan black B	lipids	-	-	-		-	±	-	±
Feulgen	DNA	-	-	-		-	++	-	++

+++ = very strong; ++ = strong; +=fairly strong; ±=medium; - = negative; α = α-metachromasia; β = β-metachromasia; γ = γ-metachromasia; * = towards the periphery in the form of a ring; ≠ = in fresh preparations strongly positive droplets; ∞ = positive areas which appear somewhat like perinuclear area; N+ = nuclear area positive; NM = not metachromatic; p = uneven.

Table 3.2

Solubility tests carried on spermatophore walls, crystalline entity and CSG secretion in O. platytarsis.

Solution	Spermatophore wall	Crystalline entity	CSG secretion	Inference
Ammonia solution 1% 50%	+	-	-	Soluble: Albumin, histones Insoluble: Chitin, fibrous proteins
Ammonium sulphate 1% 50%	+	-	-	Soluble: Histones Insoluble: Albumin, globulin, fibrous proteins, protamine, glutelin.
Sodium chloride 1% 50%	+	± clumped	-	Soluble: Globulin Insoluble: Protamine, glutelin, fibrous proteins.
Distilled water	-	-	-	Soluble: Albumin, histone, protamine Insoluble: Globulin, fibrous proteins, glutelin.
Hydrochloric acid (1% and 50%)	Coagulated	Not visible	-	Soluble: Collagen, elastin, keratin Insoluble: scleroprotein, histones Fibrous proteins
Nitric acid (1% and 50%)	Coagulated	Not visible	-	Soluble: Collagen, elastin, keratin Insoluble: scleroprotein, histones Fibrous proteins

Abbreviations: + = soluble; ± = partially soluble; - = insoluble.

blue, aqueous bromophenol blue, ninhydrin-Schiff, Millon's reagent and Sudan black B. This secretion, which is weakly PAS-positive and non-acidophilic, is rich in weakly acidic sulphated mucopolysaccharides, and may be both testicular and ductal in origin.

AVD-I gains in diameter (0.35 mm across) posteriorly. The epithelium is about 15 μm high and possesses roughly oval nuclei, about 7.5 μm along the long axis, during the active season. The luminal surface of the columnar epithelial cells is often drawn out to form a dome-shaped process giving an uneven appearance to the luminal surface of AVD-I. The epithelium of this region showed a strong positive reaction for mercury bromophenol blue and aqueous bromophenol blue (Table 3.1). However, the epithelium was not very distinct in preparations stained with ninhydrin-Schiff, alcian blue (different pH), oil red O and PAS. During the inactive phase, the epithelial cells of AVD-I were considerably reduced in size. A (glyco?) proteinaceous secretion (SE-I), basophilic and mildly PAS positive with a rather uneven texture in both paraffin sections and in vivo preparations, and apparently produced by these cells, gradually appears close to the luminal surface (Fig. 3.4) but distinctly outside the mucus-coated sperm mass. The latter on several occasions was found pushed toward the middle of the lumen. The

stainability of SE-1 is not quite stable; it showed a tendency to become acidophilic.

3.3.3.1. (b) AVD-II. AVD-I is followed by AVD-II, which could hardly be externally demarcated from the former. The secretory epithelium of AVD-II appears to be the thickest along the entire length of the anterior vas deferens of O. platytarsis. The nuclei of these epithelial cells are also seen arranged to form distinct groups; here cellular demarcations are not very distinct at the light microscope level. AVD-II has an approximate length of 1.0 cm and a width of 0.5 mm (in a crab of 5.0 cm cw). The lumen of AVD-II (Fig. 3.5) is rich in SE-I, which is distributed in between sperm masses. In in vivo preparations, the first well formed spermatophores (primary) could be detected in this region (90 ± 2.2 spermatophores per square mm). These spermatophores are elliptical to oval with a mean $\pm$ SD length of 104.29 ± 38.44 μ m and a breadth of 70.06 ± 27.48 μ m (Table 3.3). The mean $\pm$ SD number of spermatozoa per spermatophore of O. platytarsis was estimated to be 72 ± 36.13 .

Under light microscopy, the spermatophore wall of O. platytarsis appears single layered and is permeable to vital stains. Although spermatophore walls are not clearly laid in AVD-I (in comparison to posterior regions),

Table 3.3

O. platytarsis: Spermatophore size as measured in fresh preparations (expressed in microns)

<u>AVD-II/III</u> (length/width)	<u>MVD-I</u> (length/width)
157.5/94.5	126.0/81.9
81.90/63.0	63.0/44.1
126.0/37.8	107.0/75.6
69.30/63.0	56.7/44.1
157.6/126.0	81.9/63.0
126.0/94.5	189.0/126.0
94.5/69.3	75.6/69.3
132.0/63.0	88.2/63.0
157.1/63.0	37.8/37.8
113.4/81.9	69.3/44.1
176.4/126.0	63.0/50.4
69.3/63.0	69.3/37.8
63.0/31.5	81.9/63.0
63.0/31.5	88.2/56.7
176.0/63.0	88.7/50.4
81.9/56.7	75.6/56.7
94.5/94.5	69.3/56.7
94.5/81.9	88.2/63.0
126.0/119.7	69.3/50.4
63.0/63.0	63.0/37.8
75.6/44.1	44.1/37.8
63.0/37.8	63.0/37.8
63.0/37.8	75.6/44.1
113.4/75.6	69.3/37.8
69.3/69.3	63.0/44.1
Mean $\pm$ SD 104.29 $\pm$ 38.44/70.06 $\pm$ 27.48	78.64 $\pm$ 29.33/54.94 $\pm$ 19.46

beginning of their formation can be distinguished in AVD-I itself. The outermost layer of secretion surrounding the sperm mass showed an uneven staining with ninhydrin-Schiff and with PAS without oxidation. This area reacted positively with PAS (even after acetylation) and alcian blue (different pH); it stained blue with alcian blue-PAS, showing the presence of periodate-unreactive alcianophilic substances. In posterior regions, the spermatophore wall is positive to PAS, alcian blue (pH:1.0), ninhydrin-Schiff, mercury bromophenol blue, aqueous bromophenol blue and Millon's reagent, and strongly positive to alcian blue (pH:2.5). It is β -metachromatic with toluidine blue, and shows a weakly positive reaction for lipids (Table 3.1). In summary, the spermatophore wall is mucoproteinaceous in nature. Spermatophore wall of O. platytarsis is soluble in dilute and concentrated KOH and ammonia solutions and in concentrated ammonium sulphate and sodium chloride solutions (Table 3.2); chitin, thus seems to be absent in spermatophore walls. Non-acidophilic spermatophore ground substance which stains deep blue with alcian blue (pH:2.5), is practically negative to the tests for proteins and lipids. The spermatophore ground substance thus shows staining reactions comparable to those of the secretion contained in AVD-I in which are suspended the sperm masses.

Results of our histochemical investigations (Table 3.1) show that the epithelial cells in AVD-II of O. platytarsis are rich in protein: they stain positively with mercury bromophenol blue, aqueous bromophenol blue and ninhydrin-Schiff. In sections stained with Millon's reagent or PAS, it is the perinuclear zone that is more reactive. Glycogen, mucopolysaccharides and neutral lipids are rather poor in the epithelium, but with Sudan black B, a positive reaction is obtained.

3.3.3.1 (c) AVD-III. AVD-III, the third region of anterior vas deferens wider though (15 mm across) than AVD-II, is structurally continuous with AVD-II from which it is not distinguishable in external morphology. In the uncoiled state, AVD-III of O. platytarsis has an approximate length of 3.6 cm in individuals having a carapace width of 5.0 cm. The epithelia lining AVD-III and posterior part of AVD-II are rather thin ($6.5 \pm 2.35 \mu\text{m}$ in height) and share several features of common histology and histochemistry. However, in sections stained with alcian blue-PAS, the epithelium of AVD-III reveals the presence of more periodate-unreactive alcianophilic substances than periodate-reactive and non-alcianophilic substances; both types of stainable substances are absent in epithelium of AVD-II. A hyaline secretion (SE-2) makes its appearance close to the luminal surface from the posterior section of AVD-II downwards;

SE-2 is often found scattered at random amidst SE-1. A dramatic increase in quantity of SE-2 occurs further down in AVD-III (Fig. 3.6).

SE-2, a clear homogeneous, sol-like material, shows intense positive reaction to PAS (Fig. 3.26) and alcian blue (pH:1.0) (Fig. 3.22) and exhibits β -metachromasia with toluidine blue (Fig. 3.28). Protein content of SE-2 seems to be only moderate (Table 3.1). A comparison of SE-2 with the spermatophore ground substance shows that SE-2 is highly acidophilic unlike the spermatophore ground substance which is completely non-acidophilic. Furthermore, SE-2 is only weakly positive to alcian blue (pH:2.5) but spermatophore ground substance is intensely positive (Fig. 3.23). SE-2 seems to undergo some change in composition further down in the vas deferens: to alcian blue (pH:2.5) SE-2 is mildly positive in AVD-III, but completely negative in MVD (cf. Figs. 3.23 and 3.24).

With progressive increase in quantity of SE-2, SE-1 becomes relatively less prominent and arranges itself in the form of thin strands delimiting units of SE-2. The strands (?) of SE-1 on becoming thinner seem to appear as slender spindle-shaped basophilic crystalline entities (Figs. 3.6, 3.7), almost negative to PAS. In vivo observations on crystalline entities (crystal-like semitranslucent bodies) of O. platytarsis show that they

are about 20 μm long and 6 μm across in the broadest region. In fresh tissue preparations and in paraffin sections stained with haematoxylin and alcoholic eosin, the crystalline entities appear as continuous chain-like bodies, which stain deep blue. In vivo observations show that the crystalline entities are distributed in between spermatophores; in some cases they have been found attached to the spermatophore wall as well. The crystalline entities show positive reaction to aqueous bromophenol blue, mercury bromophenol blue (Fig. 3.27) and Millon's reagent, but reaction to PAS, alcian blue (pH:1.0 and 2.5), toluidine blue (Fig. 3.28) and Sudan black B was almost negative (Table 3.1). They are nearly soluble in dilute NaCl (1%) changing to a spherical shape in the process, but keep their identity intact in 1% and 50% NH_4OH (Table 3.2). These observations seem to suggest that the crystalline entities contain proteins comparable to globulin (cf. Datta and Ottaway, 1969).

Though in vivo preparations clearly show the presence of complete walls in spermatophores from AVD-II downward, the walls are not so clear when formalin-fixed, paraffin sections of AVD-II are stained. Here the spermatophores have a loose and incomplete appearance, but the primarily mucoproteinaceous and non-chitinous spermatophore wall, incomplete though, showed staining characteristics

comparable to those of well laid spermatophore walls in final form. Spermatophore walls appear incomplete in upper (anterior) regions of AVD-III also, but further down the vas deferens they become more compact and gain their final structure. From the posterior section of AVD-III downward, SE-2 organizes itself to form a number of oval or roughly spherical units, measuring 67.5 ± 33.2 μm lengthwise; each such unit of SE-2 is bordered by crystalline entities.

3.3.3.2 (a) MVD-I. MVD-I (Figs. 3.8 and 3.9) constitutes the major part of the vas deferens of O. platytarsis, being 7 cm long and 2 mm across in crabs of 5 cm cw. The epithelial wall lining the lumen of MVD-I is relatively thin (about 5.2 μm in height) and is negative to mercury bromophenol blue and aqueous bromophenol blue but positive to ninhydrin-Schiff. A weakly positive reaction results with PAS, PAS without oxidation, alcian blue (different pH), Best's carmine and Sudan black B (Table 3.1).

An appreciable increase in quantity of SE-2 and to some extent also crystalline entities occurs in MVD-I. The seminal plasma has become so abundant in MVD-I that the number of spermatophores per unit area is only about 55. The spermatophores have become smaller and more compact ($78.64 \pm 29.33 \times 54.94 \pm 19.46$ μm) in MVD-I,

compared to AVD-II/III (Table 3.3). However, compared to AVD-II/III there was no significant difference in the number of spermatozoa per spermatophore.

3.3.3.2 (b) MVD-II. MVD-II (Fig. 3.12) which is the posteriormost region of the mid vas deferens is about 1.0 cm long and 2.5 mm in diameter. Its muscular wall ($50.4 \pm 4.30 \mu\text{m}$ across) is lined by a thin epithelium. MVD-II is demarcated externally from MVD-I by a small constriction. Two stout ducts from the CSG drain their contents into the MVD-II.

Results of our histochemical observations show that the epithelium of MVD-II contains detectable quantities of protein (basic protein, tyrosyl residues, protein-bound amino groups) and lipid. The positive reaction for PAS appears to be related to 1:2 glycol groups since the original reaction is not retained after acetylation. The epithelium is only weakly positive to Best's carmine, alcian blue, and oil red O. Periodate-reactive non-alcianophilic compounds could be detected in the epithelium. As in other regions, MVD-II epithelium also is non-metachromatic with toluidine blue.

3.3.3.3 ED. In O. platytarsis, MVD-II leads to a relatively thin (0.5 mm in diameter) ejaculatory duct (ED), having an approximate length of 2 cm in crabs with a carapace



R

NB 413

595.3842

TH
SUK/c

- Fig. 3.8 O. platytarsis: a cross section through MVD-I to show the nature of its contents.
Cr - crystalline entities; S - spermatozoa
Sp - spermatophores.
Formol-saline, Heidenhain's haematoxylin-alcoholic eosin X 220
- Fig. 3.9 Spermatophores (final form)
Cr - crystalline entities, S - spermatozoa;
Sp - spermatophores.
Formalin, PAS X 290
- Fig. 3.10 Photograph of fresh preparation of ^{the} coral-shaped accessory sex gland (CSG) of O. platytarsis.
- Fig. 3.11 Fresh preparation of the semen of O. platyta showing fibrous secretion of CSG.

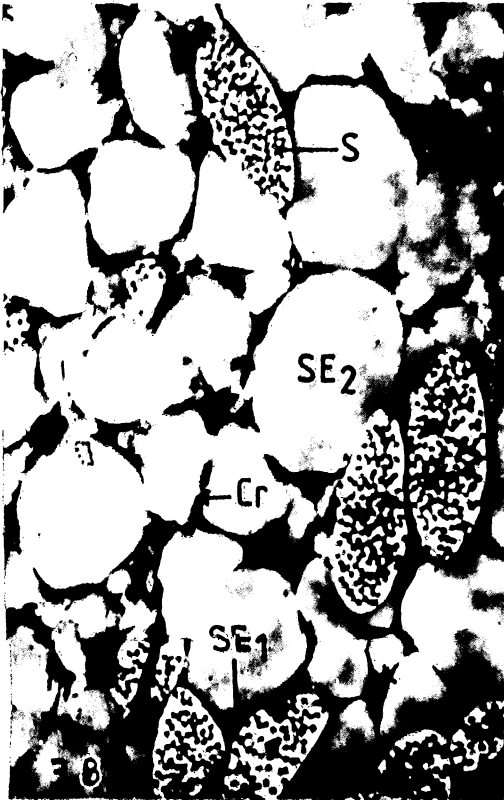
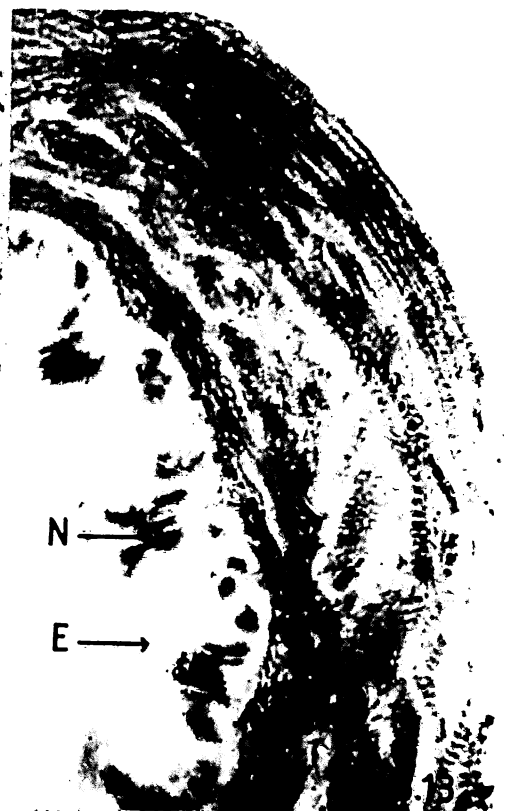
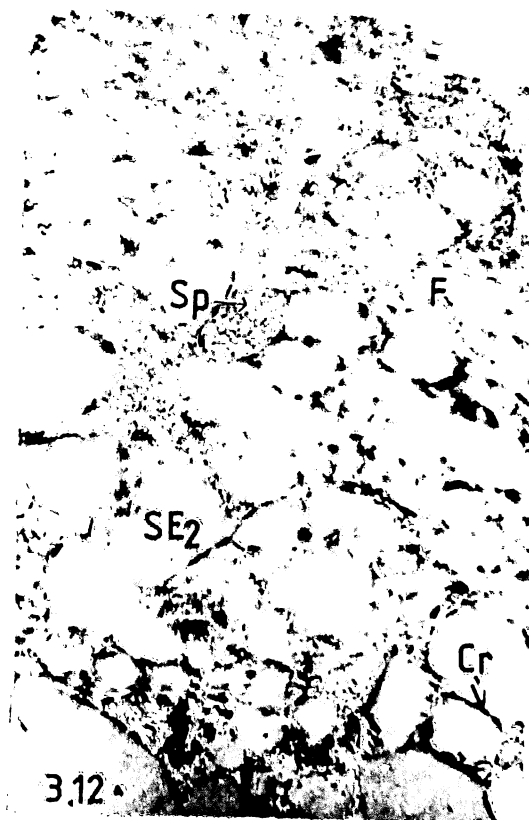


Fig. 3.12 A cross section through MVD-II of O. platytarsis.
Cr - crystalline entities; F - fibrous secretion
of CSG; Sp - spermatophores with spermatozoa.
Formol-saline, Heidenhain's haematoxylin-
alcoholic eosin X 200

Fig. 3.13 A cross section through the wall of the
ejaculatory duct of O. platytarsis.
E - epithelium; M - muscle layer; N - nuclei.
Formol-saline, Heidenhain's haematoxylin-
alcoholic eosin X 400

Fig. 3.14 A cross section through ejaculatory duct of
O. platytarsis to show remnants of semen
trapped within the lumen.
F - fibrous secretion of CSG; Sp - spermatophore with spermatozoa.
Formol-saline, Heidenhain's haematoxylin-
alcoholic eosin X 400

Fig. 3.15 A cross section through spermatheca of O. platytarsis to show the nature of its contents.
gl - gelatinous substance; S - spermatozoa.
Note the change in shape of ^{the} spermatozoa in
storage in the spermatheca.
Formol-saline, Heidenhain's haematoxylin-
alcoholic eosin X 500



width of 5 cm. The wall of ED consists of an external muscular coat and an inner layer of tall ($42.01 \pm 3.3 \mu\text{m}$) columnar epithelial cells (Fig. 3.13). The latter become evident only during the 'active' season, i.e. when the androgenic gland is in a state of high activity. We could, however, not find histological evidence for production of any secretion by ED epithelial cells. The epithelial cells stained positively with mercury bromophenol blue, aqueous bromophenol blue, Millon's reagent, ninhydrin-Schiff and Sudan black B. They were strongly positive to PAS, but the intensity of staining was found to diminish after acetylation. The possible presence of periodate-reactive, non-alcianophilic substances in these cells was indicated by alcian blue-PAS staining, but no glycogen could be detected. The epithelium was non-metachromatic with toluidine blue.

The ED^{of} each side pierces through the leg muscle bundles into a muscular penial papilla that ultimately opens into a tubular pleopod.

3.3.4. Coral-shaped Accessory Sex Gland.

The coral-shaped accessory sex gland (CSG) (Fig. 3.10), pale yellowish in colour, of male O. platytarsis is located on either side of the heart in a pocket-like depression of the thoracic muscles. In a crab having 5.0 cm cw,

the CSG measures about 10 mm antero-posteriorly and 6 mm across. Though the weight varies widely, the freshly dissected glands of an individual of 5.0 cm cw weigh about 215 mg.

In gross morphology, the gland has the appearance of a hydroid coral, with a number of short bifurcating tubules, having an approximate thickness of 0.36 mm. There is no definite branching pattern for the tubules. However, not more than one branch could be detected in a single tubule. The diameter of the tubule decreases toward the base which has a club-shaped appearance. Several such tubules of the gland finally merge to form two thick and short ducts of about 1 mm width, opening into MVD-II. Component tubules of CSG are all apparently alike and are uniformly filled with a fibrous secretion (Fig. 3.11). Each secretory fibre is of even thickness (2.0-5.3 μ m) and has a mean length of 70 μ m. In paraffin sections stained with haematoxylin and alcoholic eosin, the secretory fibres are highly eosinophilic (Fig. 3.29). The secretory epithelium of CSG tubule is moderately flat and of more or less even thickness (9.75 ± 4.99 μ m) with centrally located, highly basophilic oval-shaped nuclei ($5.1 \pm 1.9 \times 5.0 \pm 2.7$ μ m). The lumen of the CSG tubule has a striated appearance packed, as it is, fully with the fibrous secretion. However, we could not detect any

fibrous secretion in cytoplasm of the epithelial cells lining the lumen of the tubule. The mode of secretion appears to be merocrine.

Results of our histochemical investigation (Table 3.1) on the nature of the secretory product of CSG show that the protein content of the fibrous secretion of O. platytarsis is high, judging from the intensity of reaction with mercury bromophenol blue (and aqueous bromophenol blue) (Fig. 3.30). Tyrosine-containing proteins and free aldehydes were also detected. The positive reaction to ninhydrin-Schiff suggested the presence of protein-bound amino groups. The intense positive reaction to PAS, even after acetylation suggested that PAS-positive materials other than 1:2 glycol groups were also present in CSG secretion. The results of alcian blue staining revealed the presence of weakly acidic mucopolysaccharides and sulphated mucopolysaccharides in the fibrous secretion (Fig. 3.31); β -metachromasia with toluidine blue (Fig. 3.32) indicated the presence of acid groups in CSG tubules. In chilled sections, CSG tubules showed droplets positive to Sudan black B and oil red O. Histochemical analysis suggests the presence of two types of secretory fibres in CSG, one periodate-reactive and non-alcianophilic and the other periodate-unreactive and alcianophilic (Fig. 3.33).

By and large, the pattern of staining reaction of the CSG epithelium of O. platytarsis was comparable to that of CSG secretion, although the intensity of the reaction was less in the epithelium. The epithelium was non-metachromatic with toluidine blue, and weakly positive to alcian blue. Glycogen could not be detected in CSG epithelium by Best's carmine method. In PAS staining without oxidation, a positive reaction occurred in epithelial cells only in the perinuclear zone.

The secretory fibres remained unaffected on exposure to water, and to basic, acidic and salt solutions for 30 minutes (Table 3.2). In histological and histochemical preparations and in in vivo studies using methylene blue, we could not observe any difference in the nature of the fibrous secretion between CSG tubule and posterior part of the ejaculatory duct. This suggests that the CSG secretion keeps intact its nature till ejaculation.

3.3.5 Mode of Semen Production in O. platytarsis

Mature spermatozoa released from testicular acini are passed to AVD-I. Upper reaches of AVD-I are filled with spermatozoa (Fig.3.16). Definite grouping of spermatozoa into regular masses could be observed in vivo at the posterior part of AVD-I and in such preparations (stained with methylene blue) spermatozoa were found to be held together by a

Fig. 3.16 - 3.21 A diagrammatic representation of the mode of semen production in O. platytarsis.

Anterior region of AVD-I is filled with spermatozoa (Fig. 3.16). Definite grouping of spermatozoa into regular masses occurs at the posterior part of AVD-I. SE-1 is seen in between such groups. An appreciable increase in quantity of SE-1 is observed in AVD-II (Fig. 3.17), where primary spermatophores are seen. In AVD-III (Fig. 3.18) spermatophores become separated from each other and SE-2 is added to the inter-spaces. In posterior part of AVD-III and MVD-I, SE-2 is seen in greater quantity and is getting organized into specific units in between spermatophores (Fig. 3.19). Fibrous secretion of CSG is added to the primary semen as it reaches MVD-II (Fig. 3.20). In MVD-II, the spermatophores, embedded amidst units of SE-2, are generally found pushed toward the centre of the lumen. In ED, the final semen of O. platytarsis showed a characteristic arrangement (Fig. 3.21).

1 - spermatozoa, 2 - spermatophores, 3 - SE-1,
4 - SE-2, 5 - crystalline entities,
6 - fibrous secretion from CSG.

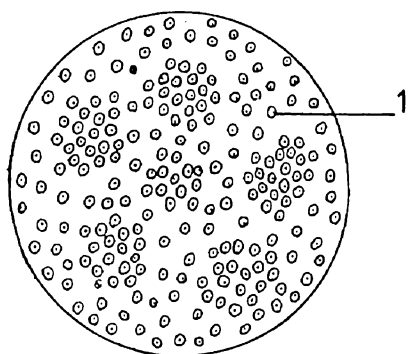


Fig. 3.16

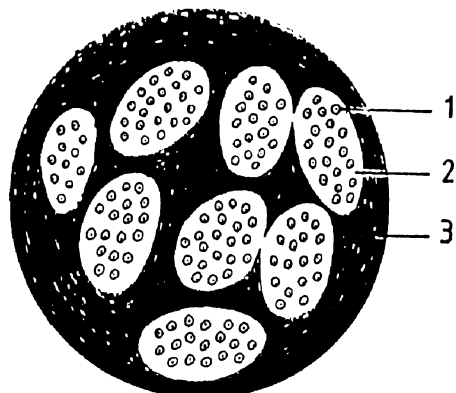


Fig.3.17

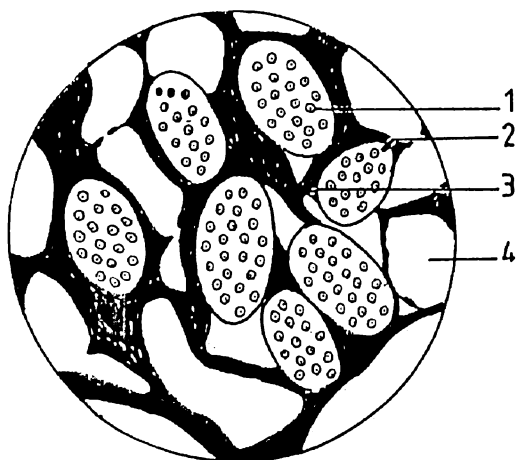


Fig. 3.18

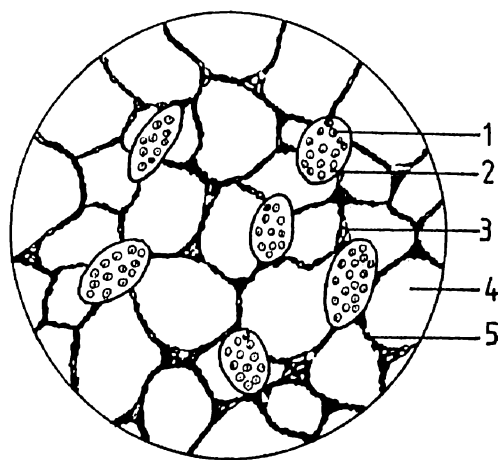
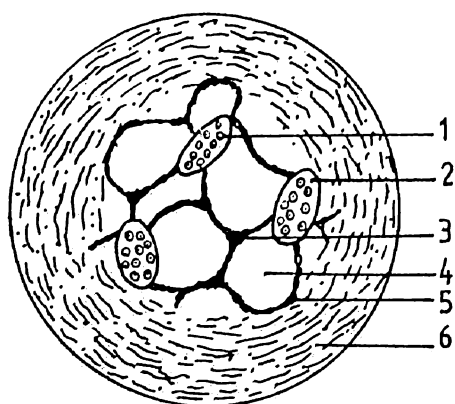


Fig.3.19



Fia . 3.20

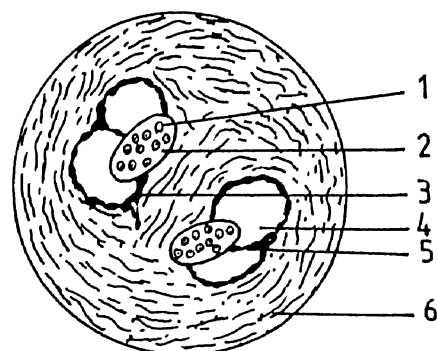


Fig. 3.21

deeply staining material. Individual spermatozoa could hardly be recognized. In between such groups of spermatozoa occurred unstained non-homogeneous material (SE-1) partially demarcating adjacent sperm groups. Corresponding pictures have been obtained in paraffin sections (stained with haematoxylin-alcoholic eosin), with SE-1 distributed in between sperm masses. SE-1, therefore, seems to play some role in grouping spermatozoa into definite masses, which may probably be the first step in semen production in O. platytarsis. As the quantity of SE-1 increased, it seemed to invade the mucus-coated sperm mass and to divide the latter into incompletely separated smaller units - the first indication of spermatophore formation. Histochemically, the secretion which holds the sperm group together is rich in weakly acidic sulphated mucopolysaccharides. Spermatophore wall was not distinct at this stage but in alcian blue (pH:2.5)- stained preparations a rather discontinuous dense accumulation (about 1.0 μ m thick) of weakly acidic sulphated mucopolysaccharides could be detected toward the periphery of each sperm mass, imparting as it were, a spermatophore wall-like appearance. As SE-1 is practically negative to alcian blue, the dense alcianophilic substance could only have originated from the mucopolysaccharide content of the sperm mass. This non-proteinaceous

alcianophilic secretion ultimately forms the ground substance of the spermatophore.

Further down in the vas deferens (i.e. AVD-II), appreciable increase in quantity of SE-1 could be observed. Each sperm mass is smaller in size, distinct and is well separated from its neighbours (Fig. 3.17). In fresh preparations stained with methylene blue, the sperm masses now have the appearance of spermatophores; only the characteristic spermatophore wall is lacking: the sperm mass is instead surrounded by SE-1. In summary, in AVD-II thickly packed spermatophores are seen. Spermatophores seem to undergo, during their passage through the vas deferens, certain changes in morphology and the final form is attained in MVD-I, where they become more compact and reduced in size, but the number of spermatozoa per spermatophore remains almost constant. More posteriorly, spermatophores become separated from each other and the space between them is filled by SE-2 (Fig. 3.18). Thus from AVD-III downwards, spermatophores are seen suspended in the lumen of the vas deferens in a medium rich in SE-2. Remnants of SE-1, which retain their glycoproteinaceous nature, could nevertheless be detected, arranged almost along the course of the crystalline entities, bordering units of SE-2. Spermatophores in the posterior region of the AVD-III

exhibit compact walls (1.0-1.2 μm thick); the spermatophore wall is conceivably formed by fusion of the crystalline entities (Section 3.3.3.1) with the discontinuous layer of weakly acidic sulphated mucopolysaccharide that had been already laid down. We are not sure if SE-2 has any definite role to play in spermatophore wall formation.

In vivo preparations show that the primary spermatophore in AVD-III is uniformly encircled by a row of crystalline entities, but in paraffin sections the encircling is not so uniform with the result that SE-2 faces directly the sperm mass within in areas where crystalline entities are lacking. It is not unlikely that the spermatophore wall might incorporate some SE-2 as well, as it attains its final structure.

In the posterior part of AVD-III and in MVD-I, the abundant SE-2 gets organized itself into specific units in between spermatophores, which become more scattered (Fig. 3.19). Therefore, the primary semen in O. platytarsis which consists of SE-2, crystalline entities, remnants of SE-1 and spermatophores (Fig. 3.8), seems to attain its final form in MVD-I, where it is stored.

One more component, i.e. the fibrous substance from CSG, is added to the primary semen as it reaches MVD-II. In this region, the fibrous secretion from CSG forms a thick layer within MVD-II, almost lining the luminal

epithelium of MVD-II internally (Fig. 3.20). The spermatophores, embedded amidst units of SE-2, are generally found pushed toward the centre of the lumen. Though occasionally spermatophores have been located within the thick duct of the CSG itself, we do not have substantial evidence to suggest that all spermatophores are normally routed through CSG before they are passed to the ED.

In O. platytarsis, the semen is not normally stored in ED, but occasionally, remnants of semen prevented from ejaculation could be detected trapped within the lumen (Fig. 3.14). In such trapped semen, different components show a characteristic arrangement: each spermatophore plus one or two units of SE-2 (bordered by crystalline entities and remnants of SE-1) is completely ensheathed by the fibrous secretion of CSG (Fig. 3.21), which forms the major part of semen. Probably, it is in this fashion that the semen is ejected into the female genital system in O. platytarsis.

3.3.6. Semen in Spermatheca

In female O. platytarsis, the ejected semen is stored in spermathecae, which are paired structures roughly oval in shape. The pale yellowish-white spermatheca of mated females of 5 cm cw is about 15 mm antero-posteriorly

Fig. 3.22 A cross section through MVD-I of O.
platytarsis.

Cr - crystalline entities; Sp - spermatophores
with spermatozoa.

Formalin, Alcian blue (pH:1.00) X 300

Fig. 3.23 O. platytarsis: a cross section through
AVD-III.

Cr - crystalline entities;
Sp - spermatophores with spermatozoa

Formalin, Alcian blue (pH: 2.5) X 290

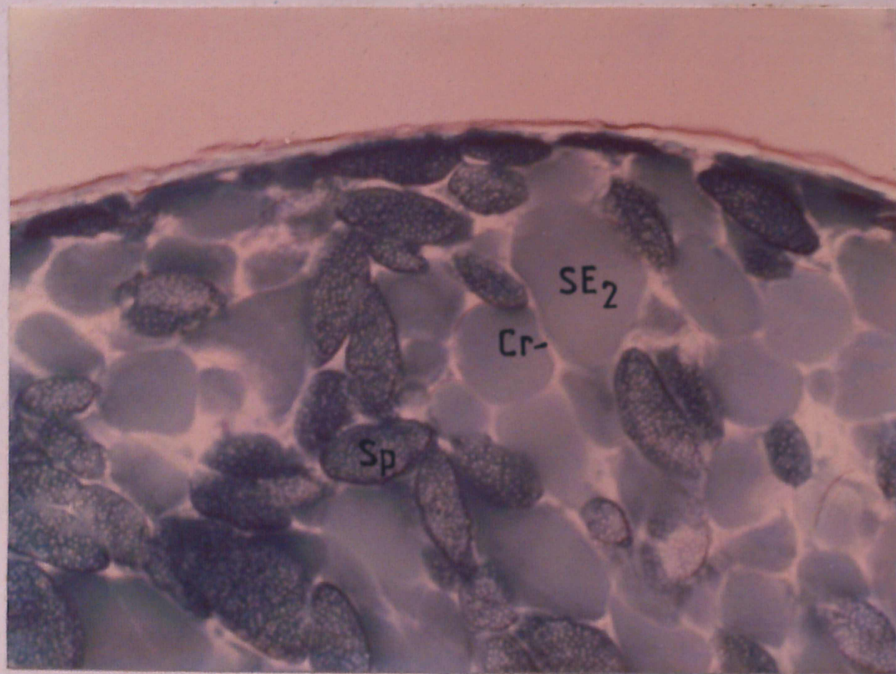


Fig. 3.22

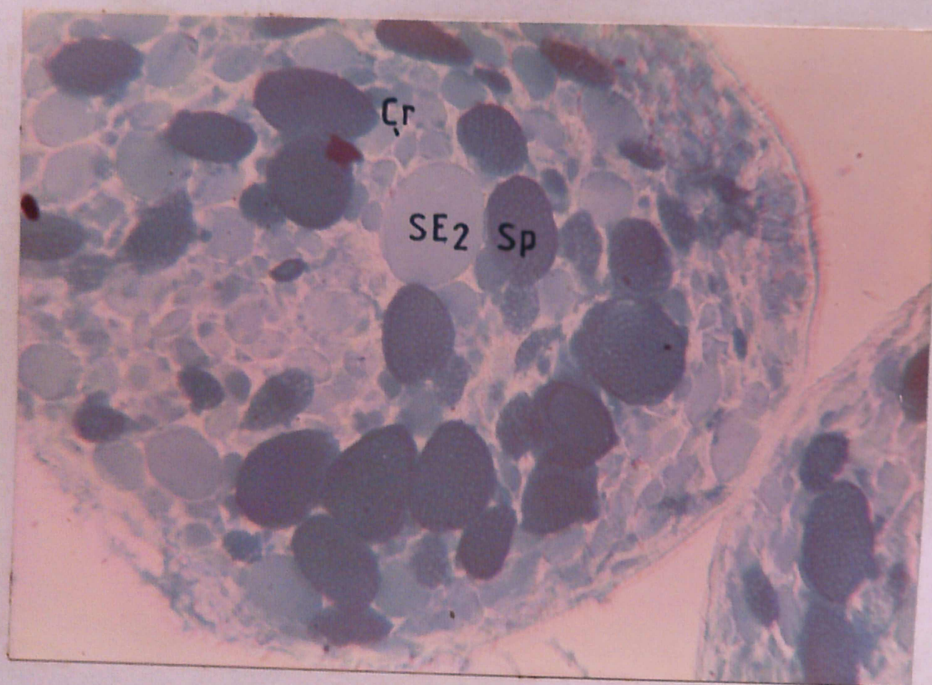


Fig. 3.23

Fig. 3.24 A cross section through MVD-I
platytarsis.

Cr - crystalline entities; Sp
with spermatozoa.

Formalin, Alcian blue (pH:2.5)

Fig. 3.25 Spermatozoa of Q. platytarsis
Formalin, Feulgen X 600

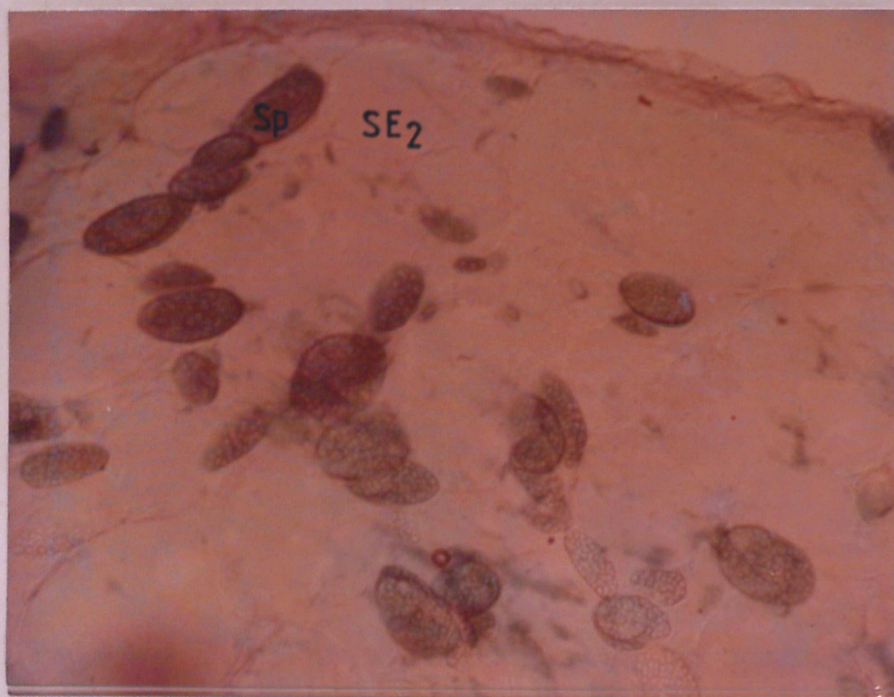


Fig. 3.24

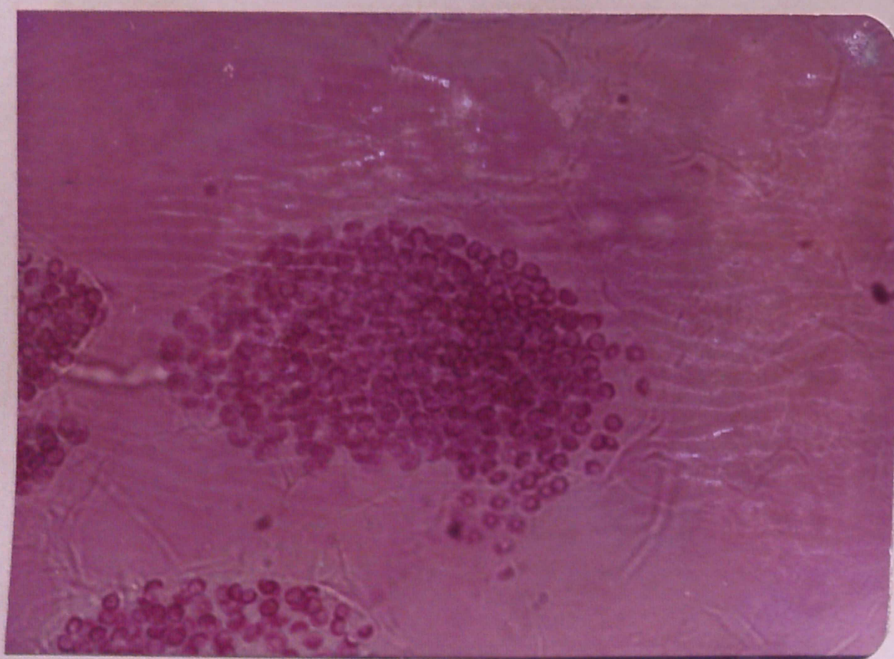


Fig. 3.25

Fig. 3.26 A cross section through MVD-I of O. platytarsis.
Cr - crystalline entities; Sp - spermatophores
with spermatozoa
Formalin, PAS X 250

Fig. 3.27 O. platytarsis: a cross section through
MVD-I.
Cr - crystalline entities; Sp - spermatophores
with spermatozoa
Formalin, Mercury bromophenol blue X 400

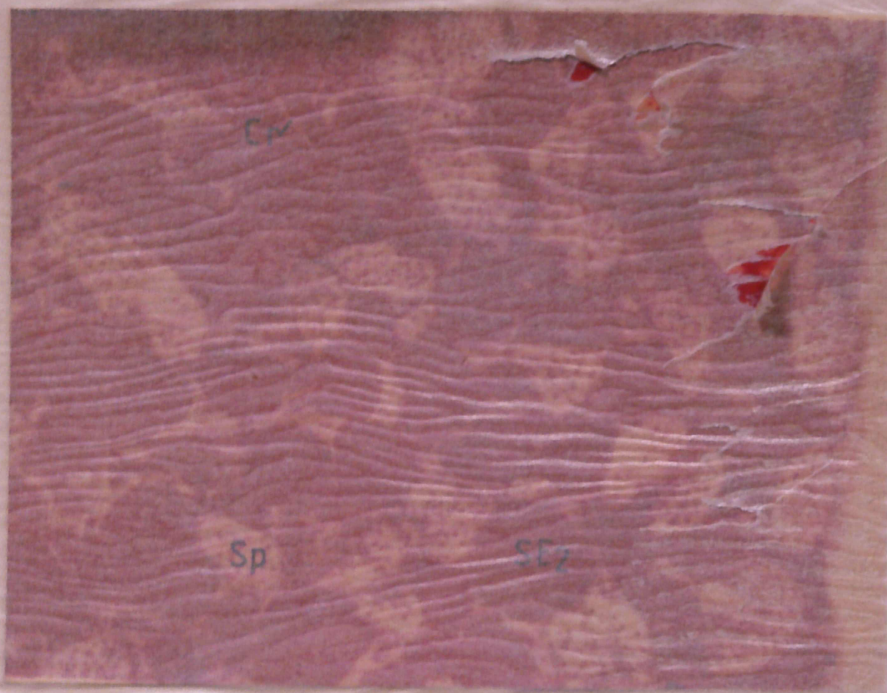


Fig. 3.26

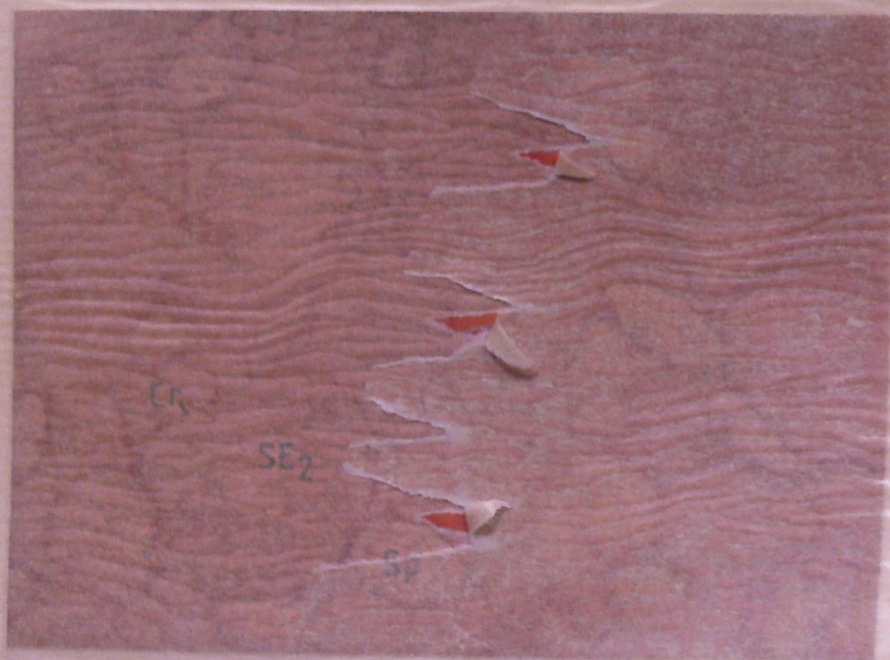


Fig. 3.27

Fig. 3.28 A cross section through MVD-I of
O. platytarsis.
Cr - crystalline entities; Sp - spermatophores
with spermatozoa
Formalin, Toluidine blue X 220

Fig. 3.29 A cross section through a CSG tubule of
O. platytarsis.
Note the fibrous secretion (F)
Formol-saline, Heidenhain's haematoxylin-
alcoholic eosin X 200

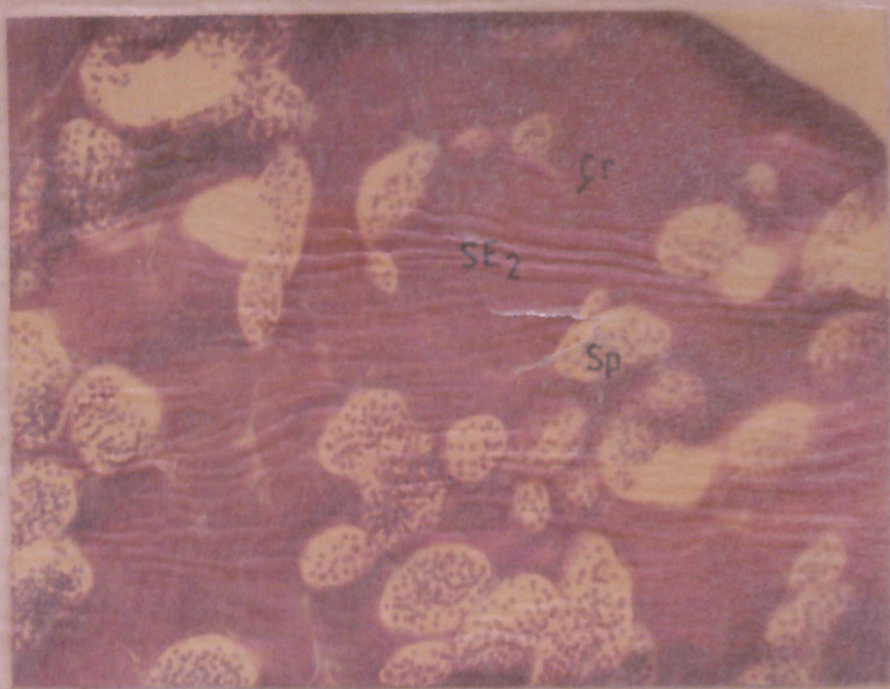


Fig. 3.28



Fig. 3.29

Fig. 3.30 A cross section through a CSG tubule of O. platytarsis showing the proteinaceous nature of the fibrous secretion (F).
Formalin, Mercury bromophenol blue X 450

Fig. 3.31 A cross section through a CSG tube of O. platytarsis showing the presence of sulphated acid mucopolysaccharides in fibrous secretion (F).
Formalin, Alcian blue (pH: 1.0) X 250



Fig. 3.30

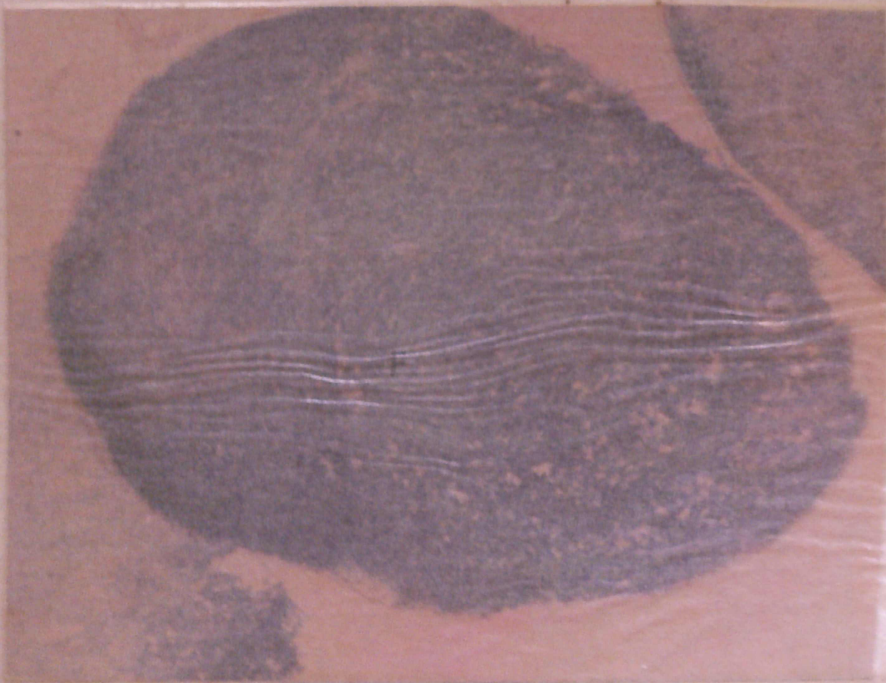


Fig. 3.31

Fig. 3.32 O. platytarsis: a cross section of the CSG tubule stained with toluidine blue. Note β -metachromasia of the fibrous secretion (F) indicating the presence of acidic substances. Formalin X 300

Fig. 3.33 O. platytarsis: a cross section of the tubule stained with PAS-alcian blue to demonstrate the existence of periodate reactive and alcianophilic substances and periodate-unreactive and alcianophilic products of fibrous secretion (F). Formalin X 450

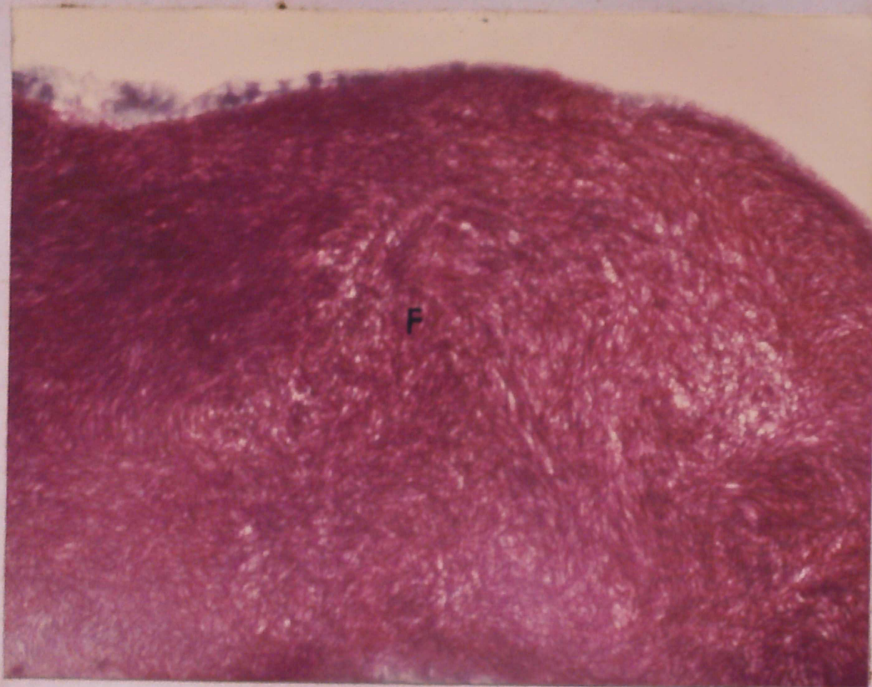


Fig. 3.32

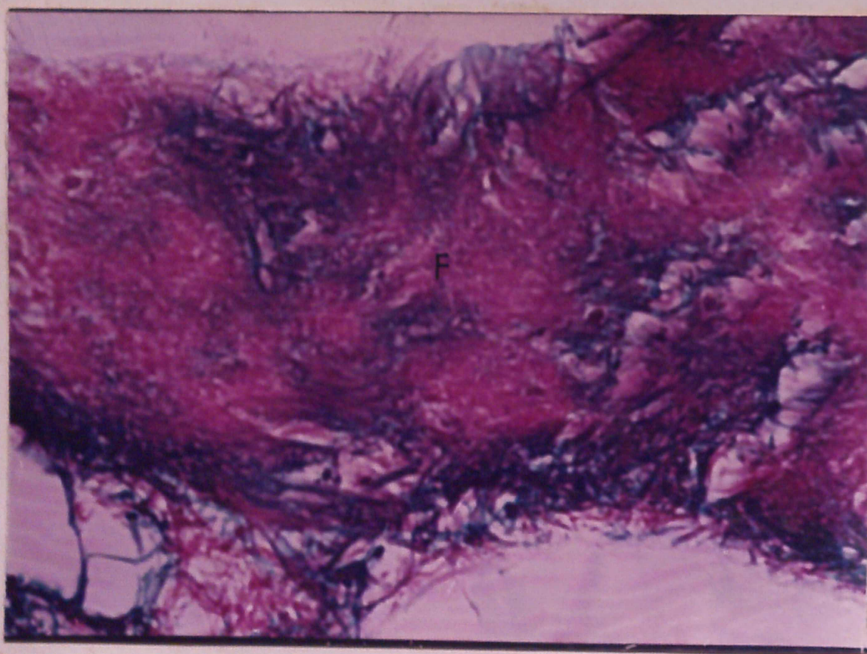


Fig. 3.33

and 8 mm across. In spermathecae of mated females, fibrous secretion of CSG was found in appreciable amounts either intact or in different stages of lysis. In paraffin sections of spermathecae stained with haematoxylin-alcoholic eosin with fibrous secretion intact, spermatophore walls were not distinct, indicating that the spermatophore walls are more easily digested than the fibrous secretion on reaching the spermatheca. The fibrous secretion of male O. platytarsis is more acidophilic than the spermathecal secretion of the female. The fibrous secretion of CSG, when fully dissolved, assumes a gelatinous appearance in which lie scattered the spermatozoa (Fig. 3.15). A change in shape of the spermatozoa also occurs in the spermatheca.

3.4. DISCUSSION

In O. platytarsis, each component of the semen (evidently with the exception of the spermatozoa) is contributed by specific gland cells arranged along the genital duct and by the CSG, a collateral accessory sex gland. As few of the seminal components accumulate as such in cytoplasm of secretory cells in light microscopically detectable proportions prior to release, the possible site of origin of each type of secretion can be only speculated based on histophysiological and histochemical evidence. The correspondence between the secretions in

the lumen and the epithelial cells of the vas deferens in staining behaviour is striking in some cases and yields valuable clue to the origin of the secretions. Thus SE-1, a secretion rich in protein, slightly positive to PAS and completely negative to alcian blue (pH:2.5) (Table 3.1), is abundant in lumen of AVD-I; the similarity in staining characteristics show by columnar epithelial cells of AVD-I shows that SE-1 may be produced by these cells and released into the lumen.

Our histological studies show that the epithelial cells lining different regions of the vas deferens of O. platytarsis vary in shape, size, nature of the nuclei and histochemical characteristics. Ultrastructural studies on the vas deferens of some crustaceans have revealed that secretory epithelial cells generally have basally located nuclei with nucleoli, a fairly well organized rough endoplasmic reticulum (RER) and sometimes smooth endoplasmic reticulum (SER), Golgi complex, mitochondria, microtubules, secretory granules and large vacuoles. Secretion produced by these cells are generally in the nature of mucopolysaccharides or mucoproteins (Pochon-Masson, 1983). The histochemical data reported here show that the glandular epithelia of AVD-II and AVD-III of O. platytarsis produce a mucoproteinaceous or mucopolysaccharide secretion. SE-2, which is a mucoprotei

sulphated acid mucopolysaccharide complex, first appears toward the periphery of the lumen in AVD-II and becomes abundant in AVD-III. Mucoproteinaceous spermatophore wall also first appears in this (AVD-II) region. Intensity of various histochemical reactions is less in epithelial cells than in secretions in the lumen. At the light microscopic level, we are not able to describe the subcellular machinery involved in synthesis of SE-2. But electron microscopic studies on 'prismatic cells' in the anterior segment of the vas deferens of Porcellio scaber by Newstead and Dronfield (1965) have revealed that the *granules* occurring in the cytoplasm are also found in the lumen of the vas deferens without an enveloping membrane. The secretory products of these cells are acid mucopolysaccharide-protein complexes. Another type of cell in the same region ('giant cell') produces a mucoproteinaceous secretion. The precursors of the proteinaceous component of the secretion, believed to be formed in association with the ribosomes encrusting cytoplasmic membranes, pass through channels of ergastoplasm to the vicinity of the Golgi complex in P. scaber. The polysaccharide component is next added and the secretion is passed out of the cell (Newstead and Dronfield, 1965). The precise mechanism by which the secretory product migrates from epithelial cell where

it is formed, to the lumen is not clearly known. It may be recalled that in cells elaborating mucopolysaccharide secretion, this mechanism is highly variable. Ultrastructural studies on isopod vas deferens seem to show that the final secretory product is transported away from the Golgi complex in vesicles. Contents of such vesicles are removed in a fashion similar to reverse micropinocytosis. The diffused pattern of distribution of presumptive acid mucopolysaccharide in cytoplasm of epithelial cells of P. scaber indicates and supports the occurrence of a system of secretion, as described above (Newstead and Dronfield, 1965). It would be interesting to investigate electronmicroscopically whether a similar type of secretory mechanism exists in epithelial cells of the vas deferens of O. platytarsis: light microscopic pictures available seem to be sufficiently suggestive that this may be the case.

The sperm-embedding secretion found in AVD-I, which ends up as the spermatophore ground substance in O. platytarsis, appears to be contributed primarily by the testis and perhaps also by the anteriormost part of AVD-I. This secretion is intensely positive to alcian blue (pH: 2.5). In the freshwater crab, Paratelphusa hydrodromous, spermatozoa are coated by a mucinous substance in testicular acini and in upper part of the

as in O. platytarsis, this material forms the ground substance of the spermatophore in P. hydrodromous (cf. Rad, 1983). The sperm embedding secretion of another freshwater crab Barytelphusa cunicularis, investigated in our laboratory, is rich in acid mucopolysaccharides. (Rad and K.G. Adiyodi, 1980). The material surrounding spermatozoa in Libinia is reported to be a mucoprotein (Schuch and Walker, 1974). Ultrastructural studies by Schuch and Walker (1974) revealed that the flocculent material surrounding spermatozoa is comparable to that found in seminiferous tubules and in spermatophores. A flocculent material of similar nature has been observed in the cytoplasm of the columnar epithelial cells of AVD (Schuch and Walker, 1974).

The origin of the crystalline entities found in semen of O. platytarsis is intriguing. We are inclined to believe that the crystalline entities may be formed from SE-1 due to some physio-chemical changes such as loss of PAS reactivity and acquisition of crystalline configuration. Material not transformed into crystalline entities continues to retain its (glyco)-proteinaceous nature, until fertilization. It seems unlikely that the crystalline entities are secreted as such by epithelial cells of the vas deferens. On the other hand, SE-2 appears to be secreted directly by the relatively thin epithelium lining the lumen of the posterior part of AVD-II downwards, possibly discharging AVD-I. At the light microscopic level, the

fibrous secretion from CSG shows its fibrous character only when the secretion is outside the epithelial cells.

Regional specificity in secretory activity has been reported in the vas deferens of many crustaceans. Histologically, the vas deferens of O. platytarsis can be divided into six different regions. The nature of the luminal contents and the cellular characteristics of the epithelium vary along the vas deferens in O. platytarsis and offer valid criteria for identification of different regions. Six regions have been described in the vas deferens of Portunus sanguinolentus (Ryan, 1967). Sperm-filled AVD-I of O. platytarsis is comparable to the proximal part of the anterior vas deferens of Paratelphusa hydrodromous (Mathad, 1983) and Libinia emarginata (Hinsch and Walker, 1974). In L. emarginata, EM studies by Hinsch and Walker (1974) have shown that spermatozoa are surrounded by a flocculent material in the anterior most region of the vas deferens; an electron-dense secretion appears toward the posterior part of the AVD which is reinforced by a muscle layer and where fully formed spermatophores are found (Hinsch and Walker, 1974). In Penaeus kerathurus, grouping of spermatozoa into definite masses takes place in the proximal (i.e., anterior) vas deferens. Multicellular glands distributed in this region secrete the sperm-embedding medium (Malek and Bawab, 1974). The 'giant cells' present in the anterior segment of the vas

of acid mucopolysaccharides. In Paratelphusa hydrodromous, a secretion consisting of basic proteins appears in between the spermatophores toward the middle part of the AVD. In more posterior regions of the AVD, certain basophilic droplets are also seen distributed in this secretion (Mathad, 1983). MVD of P. hydrodromous serves as a centre for the production of seminal plasma and for storage of the semen like MVD-I of O. platytarsis. MVD of B. cunicularis is reinforced by a distinct muscular coat outside the epithelial cells, suggesting that this region may play a role in mechanical transport of semen down the vas deferens (Mathad and K.G. Adiyodi, 1980; Mathad, 1983). In Libinia, the seminal plasma stored in MVD shows two components, a dense substance and large granules. It is suspected that MVD may absorb a secretion produced by AVD after its role (sperm grouping) has been served (Hinsch and Walker, 1974). The mid region of the vas deferens (blind pouch) of penaeus kerathurus functions as a reservoir for the storage of spermatozoa and as a secretory structure, as in O. platytarsis. In P. kerathurus, this region is subdivided into a basal blind pouch, an ascending limb and a descending limb, (Malek and Bawab, 1974). In Callinectes sapidus (Cronin, 1947) and Carcinus maenas (Spaulding, 1942), different types of secretions are produced by different parts of the vas deferens. In the brine shrimp Artemia salina, however,

the vas deferens gives almost the same histological picture throughout its length, i.e., two external muscular layers covering an internal layer of squamous epithelial cells (Wolfe, 1971). The only difference found by Wolfe (1971) in histology of the vas deferens was the increased thickness of the muscular layers toward the posterior end. It appears that in Artemia salina the whole vas deferens is involved in storage of the spermatozoa and in secretion of the seminal fluid, which may be a primitive condition.

Characteristics of the lumen of the sperm duct appear to have some bearing on the nature of spermatophores produced by Crustacea. In O. platytarsis, for example, there is a uniform increase in external diameter of the duct and internal diameter of the lumen from anterior part of AVD to posterior end of MVD. Thus AVD-III and MVD-I have a diameter greater than that of AVD-II both externally and internally, although the height of the epithelium of AVD-II is greater than that of AVD-III and MVD-I. Furthermore, the epithelial layer is rather continuous along the vas deferens which ensures a gradual increase in diameter of the lumen. As described earlier (see Section 3.3.3.1b), spermatophores of O. platytarsis are either elliptical or oval in shape and their size ranges widely (Table 3.3). Histological pictures revealing

internal morphology of the vas deferens of P. hydrodromous (Mathad, 1983) are comparable to those obtained by us on O. platytarsis, the size and shape of spermatophores being variable in both species. On the contrary, in Pagurus (Greenwood, 1972) in which the spermatophores are stalked, the columnar epithelial layer of the vas deferens is disrupted at the upper and lower borders by groove-like extension of the lumen. In Barytelphusa pulvinata which produces different types of spermatophores, the major length of vas deferens has a number of protruberances (diverticulae) on the walls, containing spermatophores (Hebbar, 1982). Lumen of the vas deferens presents a different picture in B. cunicularis (Mathad and K.G. Adiyodi, 1980), which is in keeping with its production of two types of spermatophores. Influence of mechanical forces exerted by the wall of the vas deferens and its contents in determining the size and shape of the spermatophores may only be anticipated, but more work is necessary in different crustaceans to draw meaningful conclusions on correlation between morphology of the vas deferens and morphology of the spermatophores.

The spermatophore wall in O. platytarsis is chiefly of AVD origin. In many decapods, it is the anterior part of the vas deferens that is concerned with spermatophore formation while the posterior region serves primarily for

the production of seminal plasma and storage of semen (see for example, Hinsch and Walker, 1974; Brushko, 1975). In O. platytarsis, the preliminary outline of the spermatophore wall seems to be laid by weakly acidic sulphated mucopolysaccharides produced by testis and perhaps also by secretory cells of AVD-I situated close to testis. This preliminary outline seems to be reinforced by SE-1, either directly or through crystalline entities, possibly of SE-1 origin. The role, if any, of SE-2 in spermatophore wall formation is enigmatic. The spermatophore wall becomes compact in the posterior part of AVD-III, and no further change detectable at light microscopic level occurs until ejaculation. In spermatheca, the spermatophore walls are dissolved and spermatozoa are seen distributed in the gelatinous substance resulting from dissolution of the fibrous secretion of CSG.

Spermatophore formation is usually completed in several steps. In Penaeus kerathurus, for example the main layers of spermatophores are first deposited on the sperm mass and protective layers are added on to it only at a later stage (Malek and Bawa^b, 1974). In lobsters also, a gradual thickening of the spermatophore wall is seen as the spermatophores move posteriorly in the vas deferens (Aiken and Waddy, 1981; Haley, 1984).

In O. platytarsis, spermatophores in their final form (in MVD-I) have only a monolayered wall under light microscopy. Histochemical analysis suggests that the spermatophore wall is mucoproteinaceous, and contains no chitin. In the freshwater crab P. hydrodromous also, the spermatophore wall is non-chitinous (Mathad, 1983). However, in Scylla serrata (Uma and Subramoniam, 1979), C. maenas (Spaulding, 1942), and Tisbe holothuriae (Pochon-Masson and Gharagozlou-van Ginneken, 1977), spermatophores have chitinous walls. The spermatophore wall of O. platytarsis is rich in sulphated acid mucopolysaccharides and weakly acidic mucopolysaccharides. It is soluble in ammonia solutions and is permeable to vital dyes. Spermatophore wall of Scylla serrata is double layered, the outer layer being rich in sulphated acid mucopolysaccharides (Uma and Subramoniam, 1979). Sulphated acid mucopolysaccharides may increase the permeability of the constituent layer (Fraenkel-Conrat et al., 1949; Lumsden, 1966; Morris and Finnegan, 1968). The single-layered spermatophore wall of L. emarginata is formed of a homogeneous granular material of medium electron density (Hinsch and Walker, 1974). Spermatophore walls appear to have only a relatively small role in O. platytarsis in protecting the spermatozoa from external factors such as variations in pH and salinity compared

to some other species (e.g. Dolops ranarum: Fryer, 1960) in which the spermatophores are attached externally to the females.

The various steps ultimately ending in spermatophore formation begin right from the anteriormost region of the vas deferens in O. platytarsis. The first indication is the grouping of the spermatozoa into specific groups in O. platytarsis as in Paratelpusa hydrodromous (Mathad and K.G. Adiyodi, 1979), Paratelpusa pulvinata (Hebbar, 1982) and Gecarcinus stenios (Varghese, 1984). The secretion SE-1 seems to play an important role in grouping of spermatozoa into definite masses in O. platytarsis. SE-1 first appears in lumen near the epithelial layer of AVD-I. As the quantity increases, it invades the interior of the lumen and divides the sperm mass into smaller units. In Libinia also, a secretion from the AVD is suspected to have a role in sperm grouping (Hinsch and Walker, 1974). In Penaeus kerathurus the secretion in the proximal part of the vas deferens is believed to be responsible for converting the spermatozoa into coherent masses (Malek and Bawab, 1974), while in Cancer the spermatozoa are packed into spermatophores by condensation of the secretion around them (Langreth, 1969). Except in MVD-II and in ED, no evidence for muscles could be found in O. platytarsis.

Muscles that would facilitate characteristic sperm grouping by their contraction have not been described in AVD of many crustaceans, though in some like the copepod Chondracanthus angustatus (Rousset et al., 1978), muscle fibres have been detected in this region of the male sex duct.

In all specimens of O. platytarsis examined by us in the course of this study, the upper reaches of AVD-I have been found invariably filled with spermatozoa. 'Extra-testicular activity' of the kind suggested by Wolfe (1971) or muscle contraction of AVD as suggested by Malek and Bawab (1974) cannot explain the migration of spermatozoa from the testis to the vas deferens in this species. Perhaps, the process is passive (Matthews, 1954a, b, 1957): spermatozoa reach the vas deferens because they are pushed down by a continuous wave of developing spermatozoa. The formation of spermatophores in the vas deferens of O. platytarsis appears to be largely the result of action of specific secretions on sperm masses in a phased manner, rather than the result of any muscular activity as in Pagurus (Greenwood, 1972). This is in agreement with the views of Malek and Bawab (1974) based on observations in Penaeus kerathurus and Mathad (1983) in Paratelphusa hydrodromous.

Spermatophores of O. platytarsis, notwithstanding the variations in size and shape, are all alike and belong to one type only. In Paratelphusa hydrodromous also, only one type of spermatophore has been found, but two types exist in Barytelphusa cunicularis (Mathad, 1983). In the first type of spermatophore in B. cunicularis, the ground substance is alcianophilic, whereas in the second type, alcianophilic materials are altogether absent (Mathad and K.G. Adiyodi, 1980; Mathad, 1983). In Barytelphusa pulvinata, four types of spermatophores have been described, viz. with a single core of acidophilic droplet, with numerous acidophilic droplets, with acidophilic and basophilic droplets, and without any secretory droplets (Hebbar, 1982). In Penaeus indicus, the spermatophore has a central 'C'-shaped part enclosing a basophilic secretion and spermatozoa embedded in a granular secretion; two 'wings' are also present. Spermatophores in their final form are not found in the seminal vesicle and are only formed outside the body of the male, but the various constituents of the spermatophore can be identified in different compartments of seminal vesicle. In females, the spermatophore is attached to the thelycum where the 'wings' alone are seen externally (Muthuraman, and K.G. Adiyodi, 1983; Muthuraman, 1985).

Atypical spermatozoa vary considerably in morphology

in different groups of Crustacea (Brown, 1970). The nonmotile disc-shaped spermatozoon of O. platytarsis has a central acrosome region which is PAS-positive. A PAS-positive acrosome region has been also reported in spermatozoa of P. hydrodromous (Mathad, 1983). In spermatozoa of Spirostreptus sp., the acrosomal vacuole is PAS-positive (Horstmann, 1970); a central core of PAS-positive area has been also found in spermatozoa of Calanus hyperboreus (Brown, 1970). A PAS-positive area has not been described in spermatozoa of Artemia and Chthalamus fragilis (Brown, 1970). Our histochemical studies reveal that the spermatozoa of O. platytarsis contain protein, glycogen and lipid. Spermatozoa of O. platytarsis resemble those of Paratelphusa hydrodromous in cytochemistry (Mathad and K.G. Adiyodi, 1979; Mathad, 1983). DNA (as revealed by Feulgen staining) is distributed in spermatozoa of O. platytarsis in the form of a ring; in P. indicus it may be recalled the spermatozoon has a 'half-moon shaped' nucleus and a non-metachromatic spine attached to the oval-shaped main body of the spermatozoon (Muthuraman and K.G. Adiyodi, unpublished). In the shrimp, Macrobrachium idella (Sreekumar et al., 1980), the spermatozoon is mushroom shaped with a horseshoe-like nucleus. The mature spermatozoon of M. rosenbergii resembles an everted

umbrella with a protruding spike, which is immotile. The flocculent nucleus of this species is not delimited by a nuclear membrane (Sandifer and Lynn, 1980; Lynn and Clark, 1983b). Disc-shaped spermatozoa are present in P. hydrodromous, Barytelphusa cunicularis (Mathad, 1983) and Barytelphusa pulvinata (Hebbar, 1982). In vivo observations have shown the presence of radial arms in spermatozoa of B. pulvinata (Hebbar, 1982).

The major metabolic functions of spermatozoa, viz. glycolysis and respiration, are linked to sperm motility and sperm concentration. Being immotile and rather closely packed within the spermatophore, the energy requirements of O. platytarsis spermatozoa seem to be relatively small during storage. The respiratory intensity of spermatozoa of many brachyurans is remarkably low and there is often a considerable reduction, disorganization and even disappearance of the mitochondria (Langreth, 1969; Pearson and Walker, 1975). Despite the relatively low energy requirement for survival O. platytarsis spermatozoa show appreciable quantities of glycogen and lipid reserve within them and there is no dearth of nutrients in the seminal plasma either.

O. platytarsis spermatozoa are embedded in a ground substance staining intensely for weakly acidic sulphated mucopolysaccharides and negatively for protein and lipid.

As spermatozoa are protected within spermatophores until they reach the spermatheca, this ground substance possibly has to take care of the nutritive requirements, if any, of the spermatozoa. According to Macleod (1941), acid mucopolysaccharides may function as a source of supply of reducing sugars for energy and nutrition to the spermatozoa. As the medium outside spermatophores (SE-2) in O. platytarsis has a chemical composition very different from that of the spermatophore ground substance (see Section 3.3.3.1c), it could be that the spermatophore wall may be the site of conversion of carbohydrates into weakly acidic sulphated mucopolysaccharides and reducing sugars. It is to be also examined whether the spermatophore ground substance, which in spermatozoa is 'packed' at the level of AVD itself, suffices for sperm function and survival upto their arrival in the spermatheca.

Eversion is a feature characteristic of reptantian spermatozoa. This reaction is generally shown by spermatozoa at the time of fertilization, as the spermatozoa come into contact with the surface of mature egg (Brown, 1966; Pochon-Masson, 1968a, b). Exposure to hypotonic solution also can evoke eversion reaction which is cytologically identical to that related to fertilization. In Sicyonia ingentis, the acrosome is composed of a spiraled spike and two glycoproteinaceous cap components. As the

spermatozoon comes into contact with egg, the glycoprotein-aceous component is exposed and subsequently the spike is replaced by the anterior granule in the cap which is situated proximally. Almost comparable reactions have been obtained by treating the spermatozoa with ionophore A23187 which suggests that acrosome reaction is dependent on the influx of Ca^{++} ions (Clark et al., 1981). Eversion reaction could be elicited in O. platytarsis spermatozoa by exposing them to osmotic shock. Spermatozoa right from testicular acini exhibit eversion reaction, suggesting that the ability to fertilize the eggs is acquired with or immediately following spermiation and that capacitation characteristic of mammalian spermatozoa is lacking in O. platytarsis. In P. hydrodromous, studied in our laboratory, the ability to undergo eversion reaction is absent in spermatozoa taken from testicular acini, and increases gradually from AVD to MVD, indicating that some 'ripening' of spermatozoa occurs in the vas deferens (Mathad, 1983; Krishnakumar, 1985). Comparable observations have been made on spermatozoa of Penaeus indicus (Muthuraman and K.G. Adiyodi, unpublished) and Mecrobrachium idella (Sreekumar and R.G. Adiyodi, unpublished) in our laboratory.

Regarding the fate of accessory secretions produced by the male genital system in O. platytarsis, the basophilic

secretion (SE-1), after it has played its role in sperm grouping, seems to get converted into crystalline entities. In Libinia, however, the medium electron-dense, sperm-grouping substance is resorbed in the mid vas deferens, and does not seem to undergo a chemical conversion in the lumen (Hinsch and Walker, 1974). The role of crystalline entities described by us in seminal plasma of O. platytarsis is not clear. These proteinaceous entities divide SE-2 into definite units in MVD-I. The seminal plasma of O. platytarsis is more sol-like than that of Paratelphusa hydrodromous (Mathad, 1983). Crystalline entities may have a role in regulating the flow of seminal plasma and/or in giving structural support to the more sol-like seminal plasma in O. platytarsis.

In female O. platytarsis, the spermatheca is provided with a sperm plug which is mainly made of an acidophilic secretion and has no fibrous structure (Krishnakumar, unpublished). Functionally, the sperm plug resembles SE-2. As no stainable secretory material appears to be produced by epithelial cells of ED, SE-2 seems to be directly involved in formation of the sperm plug after copulation in O. platytarsis. Interestingly in Portunus, the seminal fluid in which spermatophores are embedded and is made of these layers of materials goes to form

the sperm plug in seminal receptacle of the female (Ryan, 1967).

The males of O. platytarsis^{sex} are equipped with a well organized accessory gland (CSG) which produces the major component of the seminal plasma. CSG of O. platytarsis is not comparable to the spermatophore gland of the branchyuran Dolops ranarum, for the latter is responsible for the production of the spermatophore wall material (Fryer, 1960). The fibrous secretion of CSG is added to the primary semen comprised of SE-2, remnants of SE-1, spermatophores and crystalline entities, only at the final phase, i.e., in the region of MVD-II. Once CSG secretion reaches the spermatheca of the females, the protein-rich fibres lose their fibrous nature, possibly due to action of the spermathecal enzymes. It may be recalled here that considerable protease activity was detected in spermatheca of Ocypode ceratophthalmus (Krishnakumar unpublished). In insects, mating may serve not only in effecting transfer of male gametes to the female but also in supplying certain active principles such as fecundity-enhancing and receptivity-inhibiting substances (see Gillott, 1985 for review) to the females which play key roles in female reproductive physiology. Many male ticks also influence the physiology of mated females with the secretion of their accessory glands (Brendt and Prues, 1979).

In Calliphora vomitoria, male accessory glands are necessary for successful coupling (Errard, 1978), whereas in Rhodnius prolixus migration of the spermatozoa from the spermatophore into female by contraction of the oviduct is effected by an opaque secretion of the male accessory sex gland (Davey, 1958). Furthermore, Friedel and Gillott (1977) have suggested that accessory reproductive gland of male Melanoplus sanguinipes 'assists' female vitellogenesis and produces proteins which are incorporated antigenically intact into developing oocytes. Immunoelectrophoretic studies by Veera Raghavan et al. (1981) have revealed that in Poecillocera picta one fraction of the oocyte protein has its immunological counterpart in ASG secretion. In Squilla holoschista (Stomatopoda) some portions of male ASG secretion are possibly 'translocated' to ovary after mating (Deecaraman and Subramoniam, 1983). The cytological evidence available in O. platytarsis regarding the fate of CSG secretion in spermatheca suggests that the components present in the seminal plasma may be absorbed by the female into her system. It is not clear whether the components so absorbed have any ovary-stimulating effect in O. platytarsis as in M. sanguinipes. In any case, CSG secretion has some role in maintaining spermatozoa of O. platytarsis, embedded as they remain in modified (gelatinous) CSG secretion within the spermatheca. In ticks Ornithodoros moubata and Dermacentor

variabilis, a polypeptide (molecular weight: 12,5000) from male ASG can induce maturation of spermatozoa, after their transfer to the female (Shepherd et al., 1982).

The bulk of the accessory sex gland secretion of a number of brachyurans, unlike many insects, is not utilized for the formation of the spermatophores, but goes into the composition of the seminal plasma. The relatively small size of the brachyuran spermatophores perhaps necessitates the presence of a fluidy medium in coupious amounts for the effective transfer of a large number of spermatophores into the female system. In histological and histochemical studies, we could not, however, detect any secretory fraction other than fibrous protein in the CSG of O. platytarsis.

3.5. SUMMARY

1. Male genital system of O. platytarsis consists of a pair of testes and paired vasa deferentia which open into a penial papilla on either side through a narrow ejaculatory duct (ED). The vas deferens on each side is provided with a collateral gland - coral-shaped accessory sex gland (CSG).
2. O. platytarsis spermatozoa are disc-shaped with a distinct PAS-positive area in the middle of the

cell body. DNA is present in the form of a ring toward the cell periphery. Acrosomal reaction could be elicited in O. platytarsis spermatozoa right from testicular acini down to spermatophores within the distal end of the vas deferens. A change in shape of the spermatozoa occurs as they reach spermatheca in female O. platytarsis.

3. On the basis of histological and physiological parameters, the sperm duct of O. platytarsis has been divided by us into six regions - AVD-I, AVD-II, AVD-III, MVD-I, MVD-II, and ED.
4. Upper reaches of AVD-I are completely filled with spermatozoa passed down from the testis, probably by a passive process; here they are found embedded in a medium rich in weakly acidic sulphated mucopolysaccharides. The luminal surface of AVD-I is lined by a layer of columnar epithelial cells, the nuclei of which are arranged in groups of 2-8. SE-1, a secretion rich in proteins with mild PAS-positivity, appears toward the posterior part of AVD-I. SE-1 plays a role in grouping of spermatozoa into definite masses.
5. The secretory epithelium of AVD-II is thicker than

that of AVD-I and AVD-III. Thickly packed spermatophores (primary) are seen in lumen of AVD-II.

6. The lumen of AVD-III, which is lined by a relatively thin epithelium, contains sizeable amounts of a hyaline secretion, SE-2. SE-2, a mucoprotein-sulphated acid mucopolysaccharide complex, is highly acidophilic, clear, homogenous and sol-like in consistency. It is the medium in which spermatophores are embedded in O. platytarsis vas deferens.
7. MVD-I constitutes the major part of the vas deferens in O. platytarsis. From posterior part of AVD-III downwards, SE-2 organizes itself to form a number of oval or roughly spherical units. Each unit of SE-2 is bordered by 'crystalline entities' (crystal-like semitranslucent bodies). The crystalline entities are proteinaceous and contain little lipid or carbohydrate.
8. Primary semen of O. platytarsis, stored in MVD-I, consists of units of SE-2, crystalline entities, remnants of SE-1 and spermatophores.
9. O. platytarsis spermatophores are spherical/oval in shape. The first formed spermatophores are large and found in AVD-II; they are small and more compact in MVD-I. The nonacidophilic spermatophore

ground substance is rich in weakly acidic sulphated mucopolysaccharides, with negligible protein and lipid content. There are notable differences between spermatophore ground substance and SE-2. The single layered spermatophore walls are mucoproteinaceous and dissolve on reaching the spermatheca of female O. platytarsis. It is suggested that in O. platytarsis spermatophore formation is largely the result of action of specific secretions on sperm masses in a phased manner.

10. CSG of O. platytarsis is located on either side of the heart in a pocket-like depression formed by thoracic muscles. It has the appearance of a hydroid coral, with a number of short bifurcating tubules, all uniformly filled with a fibrous secretion. The eosinophilic secretory fibres have a high content of proteins and PAS-positive substances; glycogen and lipid droplets are also present in CSG tubules. CSG secretion retains its fibrous nature till ejaculation; it is not affected by acidic, basic and salt solutions. On reaching the spermatheca, however, the fibrous secretion dissolves forming a gelatinous substance, in which lie scattered the spermatozoa. Circumstantial

evidences suggest that CSG of O. platytarsis may have a postejaculatory role such as maintenance of spermatozoa in spermatheca.

11. MVD-II of O. platytarsis has an external muscular layer and a lining of thin epithelium internally. Two stout ducts from CSG drain their contents into MVD-II, where the fibrous secretion forms a thick layer almost lining the luminal epithelium.
12. The luminal epithelium of ED is strengthened by an external coat of muscles. Various components of the final semen of O. platytarsis show a peculiar arrangement in ED: each spermatophore with one or two units of SE-2 (bordered by crystalline entities and remnants of SE-1) is completely ensheathed by fibrous secretion of CSG, which forms the major part of the semen.

Chapter IV

Chemical Characteristics of the reproductive system and hepatopancreas of male *Ocypode Platytarsis* Milne Edwards

M Sukumaran “Certain aspects of reproductive biology of the ghost crab, *Ocypode Platyt Aris* with special reference to the male” Thesis. Department of Zoology, University of Calicut, 1985

4.1. INTRODUCTION

Information now available on chemical composition of semen in animals is uneven; the vast bulk of the literature deals with physical and chemical properties of ejaculated semen in vertebrates (see Mann, 1964; Brownwoodman and White, 1974; Arver, 1982; Daudu et al., 1983; Adamopoulos and Deliyiannis, 1983; Piironen and Hyvarinen, 1983; Sherief, 1983). Barring a few studies (Novak et al., 1960; Blum et al., 1962; Barnes, 1962a, 1963; Taber, 1977; Mathad, 1983; K.G. Adiyodi and R.G. Adiyodi, 1983 for review), our knowledge on chemical composition of semen and male reproductive system in invertebrates is far from satisfactory. This may be because ejected semen in invertebrates is much too small in volume (e.g. semen from 50 drones amounts to only 0.05 ml) for analysis compared to vertebrates, and the techniques for artificial collection of semen are only poorly developed for invertebrates. A knowledge of the chemical and physical properties of semen is nevertheless vital to studies on artificial insemination and to efforts aimed at improvement of genetic stocks of species useful to man.

In decapods, the midgut gland (hepatopancreas) is an important storage organ and possibly also a centre of intermediary metabolism. Earlier investigations in our

laboratory have indicated that hepatopancreatic reserves are mobilized in relation to moulting and reproductive activity in female crabs (R.G. Adiyodi and K.G. Adiyodi, 1970; Anilkumar, 1980). Semen production, like vitellogenesis, is a high energy-demanding process. We have studied here the biochemistry of the hepatopancreas of male Ocypode platytarsis with a view to find out whether the hepatopancreas is in a position to meet the metabolic requirements of the male reproductive system in semen production.

This chapter also gives qualitative and quantitative data on the chemistry of testis, vas deferens and coral-shaped accessory sex gland (CSG) of O. platytarsis. It complements the information gained from histochemistry of the male reproductive system (Chapter III), and allows comparison with the hepatopancreas.

4.2. MATERIALS AND METHODS

Sexually mature male Ocypode platytarsis of carapace width (cw) 5.0 to 5.5 cm were used in this study. The crabs were collected as and when needed from the shore and sacrificed suddenly without using any anaesthesia: all tests were repeated at least five times.

4.2.1. Qualitative Analysis

The testis, vas deferens (AVD+MVD-I), CSG and

hepatopancreas were carefully removed and each tissue was homogenized separately in a small quantity of distilled water. Using the homogenate, qualitative analyses were made by spot tests, as described by Feigl and Anger (1966), Levinson and MacFate (1969) and Oser (1965), and the concentrations semi-quantitated visually.

4.2.2. Quantitative Analysis

The testis, vas deferens (AVD+MVD-I), CSG and hepatopancreas of O. platytarsis were carefully dissected out in distilled water and the wet weight of each tissue was determined using a sensitive electronic balance. Excess fluid, if any, was removed by a blotting paper before taking the weight.

4.2.2.1. Proteins

For the estimation of proteins, the tissue was first homogenized in 5% trichloroacetic acid (TCA) and the precipitated protein was separated by centrifugation (1,500-2,000 rpm). The quantity of protein present was estimated by Folin's method after Lowry et al. (1951).

4.2.2.2. Free amino acids

The total free amino acids were extracted from the fresh tissue samples in 80% ethyl alcohol and were estimated after Lee and Takahashi (1966).

4.2.2.3. Carbohydrates

The oligosaccharide fraction (including free sugars) and polysaccharides were separated after Johnston and Davies (1972) and were colorimetrically estimated using the phenol-sulphuric acid method (Dubois et al., 1956).

4.2.2.4. Lipids

The estimation of total lipids was carried out by the chloroform extraction method (Vogel, 1959). The wet weight of testis, vas deferens, CSG and hepatopancreas of O. platytarsis were taken and the tissues were next dried in an oven (40°C) till the weights became constant. The total lipid in dried tissues was estimated using the Soxhlet apparatus.

4.3. OBSERVATIONS

4.3.1. Male Reproductive System

The results of qualitative analyses of the testis, CSG and vas deferens of adult male O. platytarsis are given in Table 4.1. Therefore only the salient points will be summarised here. In the male reproductive system of O. platytarsis, the testis alone showed the presence of phenylpyruvic acid and ammonia. Cysteine/cystine, inositol, citric acid, phosphates, furfural-yielding substances and furfurals were not detected in extracts of

the testis. Spot tests indicated the presence of glycogen, reducing sugars including glucose, oxalates, chlorides, steroids, sterols, phenols, acetone-bodies and creatinine in the testis of O. platytarsis. Quantitative studies showed that protein (4.57 to 7.02%) and lipids (5.26 to 7.06%) constituted the major organic constituents of the testis in terms of the wet tissue weight (Table 4.2).

Extracts of the vas deferens gave a strong positive reaction to all conventional qualitative tests for proteins. This is not surprising, for biochemical analysis (Table 4.2) showed that protein content of the vas deferens ranged from 5.45 - 18.75% of the wet weight, and protein was by far the most dominant organic constituent of the vas deferens of O. platytarsis. Some insight was also obtained on the type of proteins present in extracts of the vas deferens (see Table 4.1).

In vas deferens, the presence of amino acids and/or free amines was detected by reaction with fused potassium thiocyanate. The extract of vas deferens gave a bluish-violet colour with ninhydrin indicating the presence of free amino acids and primary amines. Total free amino acid levels in vas deferens ranged from 0.030 to 0.399% of the wet weight of the tissue. The amino acids present included cystine/cysteine (lead-blackening test) and tyrosine (Folin's test, Hoffman's reaction and formaldehyde-

H₂SO₄ test); however, no tryptophan could be detected by spot test with bromine.

The presence of reducing sugars in vas deferens is confirmed by spot test through the reduction of silver oxide. Furfural-yielding substances, pentose, mucins and glucose are found in appreciable proportions in extracts of the vas deferens; some glycogen also could be detected. Quantitative analysis indicated only low levels of carbohydrates: the percentages of oligosaccharide fraction in vas deferens ranged from 0.103 to 1.28 and of polysaccharides from 0.132 to 1.890 (Table 4.2).

The extracts of vas deferens, when heated with urea-SnCl₂-sulphuric acid mixture, produced colour only on longer boiling, indicating the presence of aldohexoses. Neutral and alkaline extracts of the vas deferens showed the presence of ascorbic acid.

In vas deferens, quantitative analysis indicated the presence of sizable amounts of chloroform-extractable lipids (1.92 to 5.49% of the wet weight of the tissue) (Table 4.2). Steroids and their esters were, however, not the major class of lipids, as shown by results of spot tests (Table 4.1).

Like the vas deferens, TCA - precipitable protein (5.30 to 14.70%) and chloroform-extractable lipids

(1.15 to 6.10%) constitute the major organic constituents of the CSG in O. platytarsis. Purdy's heat test and Robert's test suggested the possible presence of albuminous proteins in CSG as in *vas deferens*. The presence of hydroxyphenyl and phenyl groups in protein molecules was betrayed by the xanthoproteic reaction. In CSG, as in *vas deferens*, the proteins are present along with proteoses, as indicated by results of precipitation with ethanol. Tyrosine is present in fairly large amounts in CSG; cystine/cysteine also could be detected, but not tryptophan (Table 4.1). The total FAA in CSG reached a maximum value of 0.486%. The intensity of reaction shown by CSG extracts for free amines and amino acids in spot tests was somewhat higher than that shown by the *vas deferens*, which is in keeping with the quantitative data given in Table 4.2.

Reduction of silver oxide by CSG extract was intense, indicating a high content of the reducing sugars in CSG. Extracts of CSG also gave intense reaction for aldohexoses. Furfural-yielding substances, pentose, and glucose are also present in CSG. There is more oligosaccharide fraction in CSG than polysaccharide (Table 4.2).

Appreciable amounts of calcium, oxalates and chlorides have been found in CSG and *vas deferens* of O. platytarsis. The molybdic-sulphuric acid and stannous chloride test as

well as the fusion mixture test revealed the presence of phosphorus in CSG and vas deferens. Similarly, nitro-prusside (Legal's) test and Weyl's test indicated the presence of acetone-bodies in CSG and vas deferens. Nevertheless, fructose, lactose, galactose and sucrose could not be detected in these tissues. Citric acid has been detected only in the vas deferens. Methyl ketones and sulphanilic acid appear to be more concentrated in vas deferens than CSG. In the male reproductive system of O. platytarsis, inositol, however, was detected only in the CSG. O-nitrosophenol, glutathione, methionine, tertiary amines, tetrabase and aceto-acetic acid are absent in both CSG and vas deferens of O. platytarsis. Minute quantities of inorganic sulphates, p-nitrosophenols, and creatinine were indicated in CSG and vas deferens of O. platytarsis.

4.3.2. Hepatopancreas

Hepatopancreas fills almost the whole of the thorax of O. platytarsis, enveloping most parts of the digestive and reproductive systems. During intermoult, it is yellowish in colour and is provided with a number of blunt tubules. Extracts of hepatopancreas reacted rather strongly with tests for proteins. With ethanol, the extract gave white precipitate showing the presence of proteins and

proteoses. On a weight to weight basis, the hepatopaneas (7.32%) contained only as much TCA-precipitable protein as the testis (7.02%), whereas the vas deferens (18.75%) and CSG (14.70%) showed much higher protein values (Table 4.2). This deficiency in TCA-precipitable protein of the hepatopaneas was more than compensated by the free amino acid pool which at its maximum amounted to 18.25% of the wet weight of the tissue. Jaffe's test showed the presence of creatinine. Spot test with Nessler's reagent gave a pale yellow stain suggesting the presence of ammonia in hepatopaneas of O. platytarsis. Positive results with Weyl's test and Legal's test indicated the presence of acetone bodies in hepatopaneas; the presence of sterols (cholesterol) and their esters, methylketones, citric acid, sulphanilic acid, calcium, phosphates, phosphorus, oxalates, inorganic sulphates and chlorides was also indicated in hepatopaneas by various tests. Reducing sugars, furfural-yielding substances, sucrose, glucose, pentose, aldohexoses, inositol and phenols could be detected in hepatopaneas. However, substances such as O-nitrosophenol, galactose, lactose, tryptophan and tetrabase have not been found either in the hepatopaneas or in the reproductive system (Table 4.1) of O. platytarsis.

4.3.3. Primary Semen

In O. platytarsis, the contents of MVD-I which can

Sl. No.	Experiments	Testis	Coral-shaped gland (CSG)	Vas deferens (VD)	Hepato-pancreas (HP)	Inferens
I	II	III	IV	V	VI	VII
1.	Test for reducing substances with phosphomolybdic acid	blue colour (+)	blue colour (+)	blue colour (+)	blue colour (+)	blue colour reducing substances present
2.	Spot test through transformation of furfurals	light pink stain on the reagent paper (+)	deep pink stain on the reagent paper (+)	deep pink stain on the reagent paper (++)	deep pink stain on the reagent paper (+++)	carbohydrates present
3.	Detection of reducing sugars through silver oxide reduction	black flake of silver on the stain (+)	black flake of silver on the stain (+++)	black flake of silver on the stain (++)	black flake of silver on the stain (+++)	reducing sugars possibly present
4.	Picric acid test for reducing sugars	ND	light mahogany red (±)	ND	mahogany red colour (+)	reducing sugars in CSG and HP

contd...

I	II	III	IV	V	VI	VII
5.	Molisch test (α -naphthol reaction)	ND	brown ring (++)	brown ring (+)	brown ring (++)	furfural or furfural-yielding substances present in CSG, VD, HP
6.	Condensation with aniline	ND	ND	ND	ND	native furfurals not detected
7.	Spot test with pholoroglucinol	ND	ND	ND	ND	native furfurals not detected
8.	Benedict's test (reducing sugars)	no preci- pitate	white turbid	bluish white precipitate	yellowish white preci- pitate	reducing and non- reducing sugars present
9.	Benedict's test after acidifying with con. HCl and neutralizing with Na_2CO_3 solution	ND	ND	ND	vivid green	HP contains sucrose
10.	Barfoed's test (mono-and disaccharides)	pale green precipi- tate	pale green precipi- tate	pale green precipi- tate	pale green precipitate	red precipitate indicated mono- and disaccharides

contd...

	I	II	III	IV	V	VI	VII
11. Bial's test			pale green (+)	green (+)	green (++)	green (+++)	positive reaction indicated the pre- sence of pentose
12. Mucic acid test		ND	ND	ND	ND	ND	lactose and gala- ctose not detected
13. Rubner's test (modified for lactose)		ND	ND	ND	ND	ND	lactose not detected
14. Spot test for aldohexoses		ND	ND	on longer boiling colour developed (++)	on longer boiling colour developed (++)	on longer boiling colour developed (++)	positive test indi- cates aldohexoses
15. Resorcinol-HCl reaction (Seli- vanoff test)		ND	ND	ND	ND	ND	fructose not detected
16. Test with iodine solution		wine red colour (++)	wine red colour (+)	wine red colour (+)	wine red colour (+)	wine red colour (+)	presence of glycogen
17. Jaffe's test for glucose		light red colour (+)	bright red colour (++)	bright red colour (++)	bright red colour (++)	bright red colour (++)	presence of glucose

contd...

I	II	III	IV	V	VI	VII
---	----	-----	----	---	----	-----

18. Detection of inositol through oxidation with HNO_3	ND	reddish brown (+++)	ND	reddish brown (+++)	positive reaction indicates the presence of inositol
19. Spot test by reduction of ammonium-phosphomolybdate:					
a) using neutral tissue extract	blue colour (+)	?	blue colour (+)	blue colour (++)	positive reaction indicates the presence of ascorbic acid
b) using acidified tissue extract (HCl)	ND	ND	ND	blue colour (?)	ascorbic acid is below the level of detection by this method
c) using alkaline extract (NaOH)	?	?	dark blue colour (+)	dark blue colour (+)	possible presence of ascorbic acid
20. Spot test with nitrous acid	light brown colour (+)	brown colour (+)	brown colour (++)	brown colour (++)	presence of phenols
21. Spot test by formation of cobaltic chelate compounds	ND	ND	ND	ND	Q-nitrosophenols absent
				contd...	

II		III	IV	V	VI	VII
Spot test with thymol- H_2SO_4		?	violet colour changes to blue with ammonia	violet colour changes to blue with ammonia	?	possible presence of p-nitrosophenol in CSG and VD
Heat tests (proteins):						
a) Boiling with water		white turbid	white coagulant	white coagulant	white coagulant	proteins present
b) Heat with 33% acetic acid		faint white cloud	white cloud	white cloud	white cloud	proteins present
c) Purdy's heat test		white cloud	white cloud	white cloud	white cloud	presence of albumin
Nitric acid- $MgSO_4$ ring test (Robert's test for albuminous proteins)		solutions are stratified by a pale yellow precipitate	solutions are stratified by a pale yellow precipitate	solutions are stratified by a pale yellow precipitate	solutions are stratified by a pale yellow precipitate	"
Precipitation with metallic salts:						
a) $HgCl_2$ solution		white turbid	white precipitate	gray precipitate	yellowish white precipitate	presence of proteins

contd...

I	II	III	IV	V	VI	VII
	b) $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2$ solution	white precipitate	white precipitate	white precipitate	yellowish white precipitate	presence of proteins
	c) CuSO_4 solution	blue precipitate	bluish white precipitate	bluish white precipitate	bluish white precipitate	”
	d) AgNO_3 solution	white turbid	white precipitate	gray precipitate	yellow precipitate	”
	e) BaCl_2 solution	white precipitate	white precipitate	white precipitate	white precipitate	”
26.	Precipitation with other reagents:					
	a) Phosphotungstic acid	white turbid	white precipitate	white precipitate	white precipitate	”
	b) Phosphomolybdic acid	pale green turbid	pale green precipitate	pale green turbid	pale yellow precipitate	”
	c) Trichloroacetic acid (TCA)	white precipitate	white precipitate	white precipitate	white precipitate	”
27.	Extol's test	white cloud	white precipitate	white precipitate	white precipitate	”

contd...

I	II	V				VII
		III	IV	V	VI	
28.	20% sulphosalicylic acid	white turbid	white precipitate	white turbid	white precipitate	presence of proteins
29.	Precipitation with ethanol	white turbid	white turbid	white turbid	white turbid	proteins along with proteoses present
30.	Biuret test	pinkish violet colour	pinkish violet colour	pinkish violet colour	pinkish violet colour	presence of proteins
31.	Nitroprusside reaction	ND	ND	ND	ND	glutathione absent
32.	Ninhydrin reaction	blue colour (+)	blue colour (++)	blue colour (++)	blue colour (+)	<i>proteins, peptones, peptides, free- amino acids, primary amines or ammonia may be present</i>
33.	Spot test by reaction with fused potassium thiocyanate	brownish black colour on the reagent paper (++)	brownish black colour on the reagent paper (+++)	brownish black colour on the reagent paper (++)	brownish black colour on the reagent paper (+++)	presence of amino acids or free amines

contd...

I	II	III	IV	V	VI	VII
34. Millon's reaction						
		reddish brown precipitate	reddish brown precipitate	reddish brown precipitate	reddish brown precipitate	hydroxyphenyl group ($-C_6H_4OH$) in proteins (tyrosine, halogenated tyrosine or 3-, 5-unsubstituted phenol present)
35. Hofmann's reaction (Millon's test modified by Cole)						
	ND	reddish brown precipitate	reddish brown precipitate	reddish brown precipitate	reddish brown precipitate	tyrosine may be present in CSG, VD and HP.
36. Folin's test						
	blue colour (+)	blue colour (++)	blue colour (++)	blue colour (+++)	blue colour (+++)	presence of tyrosine
37. Formaldehyde- H_2SO_4 test for tyrosine						
	?	pale green colour	pale green colour	pale green colour	yellowish green colour	"
38. Xanthoproteic reaction						
	slight yellow	light orange	light orange	light orange	light orange	presence of phenyl group ($-C_6H_5$) in the protein molecule
39. Spot test with bromine						
	ND	ND	ND	ND	ND	tryptophan not detected
40. Lead-blackening test						
	ND	brown solution with black precipitate	brown solution with black precipitate	brown solution with black precipitate	brown solution with black precipitate	presence of cysteine/tyrosine in CSG, VD and HP

contd...

I	II	III	IV	V	VI	VII
41. Bolling's modification of McCarthy-Sullivan test	ND					
42. Test with sodium nitroprusside for -SH groups	ND	ND	ND	ND	ND	methionine not detected
43. Test with FeCl ₃ and CuSO ₄	ND	ND	ND	ND	ND	-SH groups not detected by this method.
44. Spot test with citric acid and acetic anhydride	ND	ND	ND	ND	ND	"
5. Precipitation with acetic acid	clear solution	ND	white turbid	ND	ND	tertiary amines not detected
46. Salkowski test (for sterols)	pale green in acid layer	pale green in acid layer	pale green in acid layer	pale green in acid layer	pale green in acid layer	<i>mucoproteins, glycoproteins, nucleoproteins, and the like present in CSG, VD and HP.</i>
47. Liebermann-Burchard test	bluish green colour (++)	bluish green colour (+)	bluish green colour (+)	bluish green colour (+)	bluish colour	sterols may be present possible presence of steroids/esters

contd...

I	II	III	IV	V	VI	VII
48.	H ₂ SO ₄ - formaldehyde test for cholesterol	light cherry-red colour (+)	?	?	cherry-red colour (+)	possible presence of cholesterol in testis and HP
49.	Jaffe's test	red colour turns into yellow (+)	red colour turns into yellow (+)	red colour turns into yellow (+)	red colour turns into yellow (+)	possible presence of creatinine
50.	Test with phenolphthalein	ND	ND	ND	ND	ammonia not detected by this method.
51.	Test with Nessler's reagent	orange stain	ND	ND	yellow stain	presence of ammonia in testis and HP
52.	Test with manganese dioxide	ND	ND	ND	ND	tetrabase not detected
53.	Nitroprusside (Legal's) test	orange-chrome	orange-chrome	orange-chrome	orange-chrome	possible presence of acetone bodies
54.	Nitroprusside test modified by Oser et al. (1965)	yellow ring	yellow ring	yellow ring	yellow ring	possible presence of acetone bodies
55.	Weyl's test	orange-chrome	orange-chrome	orange-chrome	orange-chrome	"
56.	Rothera's test	ND	ND	ND	ND	acetone/acetone-acetic acid not detected

contd...

I						
II						
III						
IV						
V						
VI						
VII						
57. Gerhardt's test	ND	ND	ND	ND	ND	acetoacetic acid absent
58. Gunning's idoform test	ND	ND	ND	ND	ND	acetone absent
59. Spot test with sodium-nitroprusside	ND	yellow colour turns to light blue (+)	yellow colour turns to blue (+)	yellow colour turns to blue (++)	yellow colour turns to blue (+)	presence of methyl ketones
60. Spot test through conversion into pentabromoacetone	ND	ND	ND	ND	ND	acetone absent
61. FeCl_3 (Meuleman's test)	ND	pale green	ND	white precipitate	white turbid	VD and HP contain citric acid
62. Spot test through the release of H_2SO_4	ND	ND	ND	ND	?	testis contains phenylpyruvic acid
63. Salkowitch test	white precipitate	white turbid	white precipitate	white precipitate	white precipitate	presence of sulphanic acid
64. Test with NH_3 and oxalate	turbid	turbid	white precipitate	white precipitate	yellow precipitate	presence of calcium
65. Test for phosphates with HNO_3 and ammonium molybdate	ND	yellow turbid	turbid	turbid	white precipitate	"
					yellow precipitate	presence of phosphates

contd...

Table 4.2.

Quantitative estimation of the major biochemical components of the testis, vas deferens, coral-shaped accessory gland (CSG) and hepatopancreas of male Ocypode platytarsis M. Edwards (Quantity expressed in mg/100 mg of wet tissue weight).

Components	Testis	CSG	vas deferens	Hepato- pancreas
<u>Proteins</u>				
Maximum	7.02	14.70	18.75	7.32
Minimum	4.57	5.30	5.45	0.88
<u>Free amino acids</u>				
Maximum	3.02	0.486	0.399	18.25
Minimum	0.15	0.021	0.030	0.097
<u>Oligosaccharide fraction</u>				
Maximum	0.87	1.26	1.28	1.79
Minimum	0.208	0.124	0.103	0.13
<u>Polysaccharides</u>				
Maximum	0.33	0.562	1.890	1.324
Minimum	0.246	0.020	0.132	0.042
<u>Lipids</u>				
Maximum	7.06	6.10	5.49	28.13
Minimum	5.26	1.15	1.92	12.75

be collected by cutting and gently pressing the duct make the primary semen. The primary semen has a dull white colour. It is viscous fluid with a density of 1.29. The mean content of TCA-precipitable proteins in primary semen of O. platytarsis is calculated to be about 164 mg/ml. The corresponding value for FAA is 1.16 mg/ml, for oligosaccharide fraction 7.72 mg/ml, and for polysaccharide 2.82 mg/ml. The final semen is a combined product of the primary semen and secretions of the CSG and is the form in which is ejected at the time of copulation.

4.4. DISCUSSION

The physical characteristics of the primary semen (from MVD-I) of O. platytarsis such as colour and density are comparable to those of the semen of the freshwater crab, Paratelphusa hydrodromous investigated in our laboratory (Mathad, 1983). For example, O. platytarsis primary semen has a density of 1.29 which compares favourably with the density (1.23) of P. hydrodromous semen (Mathad, 1983). Semen of crabs appears to be more dense than seminal plasma of the barnacle Balanus balanus which has a density of only 1.041 to 1.044 (Barnes, 1962a); mammalian semen exhibits a still lower density. As in other animals, proteins form the most prominent organic constituents of primary semen (contents of MVD-I) of

O. platytarsis. Compared to B. balanus (Barnes and Blackstock, 1974), man and other domestic animals (see Mann, 1964 for details), the protein content of the primary semen of O. platytarsis is rather high (164 mg/ml). Relatively high protein values (144.96 - 233.00 mg/ml) have been also recorded for the semen (collected from MVD) of P. hydrodromous (Mathad, 1983). The final semen of O. platytarsis contains sizable amounts of fibrous, highly proteinaceous secretion of CSG origin; protein content of the ejected semen is therefore likely to be even higher than that of primary semen. The relatively high protein content of the semen of P. hydrodromous is morphologically related to the abundance of protein-secreting gland cells in its vas deferens epithelium and of O. platytarsis in both vas deferens epithelium and CSG. According to R.G. Adiyodi (1985, personal communication), this difference in protein content of semen in crustaceans may be physiologically related to difference in their fertilization biology: species resorting to external fertilization (e.g. Balanus balanus, Macrobrachium idella) produce semen containing only small amounts of protein, whereas species resorting to internal fertilization produce semen rich in proteins. Seminal chemistry of more species has to be studied to confirm this generalisation.

Table 4.2 shows that the protein content of the male reproductive system of O. platytarsis varies in different regions (testis, vas deferens, CSG). On a weight to weight basis, the vas deferens shows the maximum percentage of TCA-precipitable proteins, followed by CSG. This observation is in keeping with the highly proteinaceous nature of the primary and final semen. Results of qualitative tests indicate that nitrogenous products in semen may include peptides, proteoses, amino acids and primary amines. Histochemical observations reported earlier in Chapter III on semen of O. platytarsis have been generally confirmed by qualitative tests listed in Table 4.1. Seminal proteins of O. platytarsis have not been fractionated electrophoretically, but qualitative tests reveal that they contain $-C_6H_4OH$ and $-C_6H_5$ groups. Besides tyrosyl residues, cystine/cysteine residues have been also detected, and indications are that one or more species of seminal protein may be muco- or glyco-protein. At the histochemical level, components of semen rich in protein are the crystalline entities, fibrous secretion of CSG, and of course also the spermatozoa.

Seminal proteins in ejaculated semen of O. platytarsis undergo enzymatic digestion resulting in increased production of peptides and free amino acids. This is particularly apparent in semen transferred to the

spermatheca of female O. platytarsis following copulation (Krishnakumar, 1985). The protease is produced by the spermatheca. It is possible that breakdown products of seminal proteins in spermatheca may be used by the female to replenish her protein (nitrogen) requirements associated with vitellogenesis (R.G. Adiyodi, 1985, personal communication).

Quantitative analysis suggests that the total FAA in the male reproductive system of O. platytarsis undergoes fluctuations, and that the testis has more FAA than other parts of the male reproductive system (Table 4.2). In P. hydrodromous also, the AVD and semen showed great variations in FAA (Mathad, 1983). However, in Balanus balanus, amino acids of seminal plasma remained much the same throughout the sampling period (Barnes and Blackstock, 1974).

The functional significance of free amino acids (FAA) in semen has been the subject of much discussion. Tyler and Rothschild (1951) believe that the beneficial action of the amino acids is due primarily to their metal-binding capacity. The investigations by MacLeod (1946, 1951) have shown that cysteine and glutathione protect human spermatozoan motility and glycolysis from harmful effects of spermicidal agents like copper ions. According to

Shannon and Curson (1983), cysteine enhances sperm survival in a reduced air saturation. Amino acids also function as a buffer against the adverse effects of dilution and can induce maximum utilization of the endogenous substrates (Mann, 1964). FAA are also considered as an extracellular oxidizable substrate for the aerobic metabolism of spermatozoa. Studies by Bhargava *et al.* (1959) on bull semen have revealed that its amino acid content is derived mainly from seminal plasma, rather than from spermatozoa. It has been further suggested that the spermatozoa are capable of incorporating FAA from their extracellular environment (Bhargava, 1957; Bhargava *et al.*, 1959). Glutamic acid is the predominant amino acid in semen of ram and bull, whereas glycine is predominant in human seminal plasma (Brown-woodman and White, 1974). Spermatozoa can oxidise L-tyrosine, L-phenylalanine and L-tryptophan (Tosic and Walton, 1945, 1946, 1950); furthermore, respiration of spermatozoa can be increased by the addition of various amino acids (Iida and Kawai, 1957). The present study shows that the protein and FAA contents of O. platytarsis vas deferens and CSG are rather high and also flexible. Possibly, FAA resulting from breakdown of seminal proteins by enzymic digestion in spermatheca of

O. platytarsis may be utilized by spermatozoa as a substrate to ensure survival, for semen is stored in spermatheca for long periods in preparation for fertilization.

Meuleman's test with FeCl_3 shows that phenylpyruvic acid is present only in testis of O. platytarsis (Table 4.1). In animal tissues, phenylpyruvic acid can be converted to phenylalanine, an essential amino acid in mammals, and metabolized through tyrosine (West et al., 1966)

Aerobic and anaerobic incubation of semen leads to hydrolytic degradation of proteins and enzymic deamination of amino acids, and to production of ammonia. The fresh semen of many animals also contains ammonia. Almost comparable amounts of ammonia (2 mg/100 ml) have been detected in the semen of man (Mann, 1964), bull (Shergin, 1933) and ram (Mann, 1964). In O. platytarsis, the extracts of only the testis showed the presence of ammonia.

Barnes (1963) reported that during spermatogenesis some proteolysis occurred and the testis alone contained the responsible enzymes. Moreover, spermatocytic development was always associated with characteristic changes in amino acids and nucleoproteins.

Vas deferens and CSG of O. platytarsis contain the nitrogenous compound, creatinine. Creatinine has been

also detected in the vas deferens of the Indian prawn Penaeus indicus (Muthuraman and K.G. Adiyodi, 1984).

Creatinine content of the seminal vesicle of boar is more than that of its blood plasma (McKenzie et al., 1938).

Bull semen and stallion semen also contain appreciable quantities of creatinine (Ilyasov, 1933). Testis and spermatozoa of many invertebrates contain creatinine.

Creatinine is usually formed when the energy-rich compound creatine phosphate is degraded to form creatine, phosphate and energy; the latter gets coupled to ATP synthesis.

Creatine is next converted to creatinine. What role creatinine plays in semen is not understood.

Coming to carbohydrates, spot tests (Table 4.1) indicated the presence of reducing sugars and other reducing substances in vas deferens and CSG of O. platytarsis. Biochemical analysis (Table 4.2) has also shown the presence of moderate amounts of carbohydrates (oligosaccharide fraction and polysaccharides) in male reproductive system. Fructose, which is the main seminal sugar in semen of many invertebrates and vertebrates has not been detected either in the vas deferens or in the CSG of O. platytarsis. But glucose, pentose, glycogen and aldohexoses have been distinguished. Vas deferens of Penaeus indicus (Muthuraman and K.G. Adiyodi, 1984) contains only negligible amounts of fructose but more glucose and trehalose. Three free

sugars - glucose, fructose and trehalose - have been reported in reproductive system of the honey bee, Anis mellifera, although their concentrations vary in different reproductive organs. Testis is the richest source of reproductive carbohydrates in A. mellifera. After the flight, most sugars in reproductive system of this species are depleted. The penis bulb contributes the bulk of the seminal sugar during ejaculation (Blum et al., 1962; Taber, 1977 for review). These sugars (except trehalose) could induce spermatozoan motility when added to saline solution (Poole and Edwards, 1972).

Table 4.2 shows that among carbohydrates, on a weight to weight basis, the oligosaccharide fraction is more dominant than the polysaccharide fraction in testis, CSG and hepatopancreas, but not in the vas deferens which has more polysaccharide than oligosaccharide. The total carbohydrate content (oligosaccharide fraction + polysaccharide) of the primary semen of O. platytarsis is calculated to be about 10.54 mg/ml (Section 4.3.3). In the freshwater crab, P. hydrodromous, the maximal carbohydrate content of semen is 4.56-6.0 mg/ml (Mathad, 1983) while that of cirripede seminal plasma is only 2.0 mg/ml (Barnes and Blackstock, 1974). In the semen of Penaeus indicus (Muthuraman and K.G. Adiyodi, 1984) the oligosaccharide fraction is as high as 30.22 ± 0.7116

mg/ml and polysaccharide fraction 28.867 ± 2.661 mg/ml. These observations show that the quantity of seminal carbohydrates is subject to much variation between species and within the species themselves. Mucins, glycogen and furfural-yielding substances were also detected by spot tests in vas deferens and CSG of O. platytarsis. Our earlier histochemical findings in the same tissues of O. platytarsis have already confirmed the presence of acid mucopolysaccharides, glycogen, and other PAS-positive substances (see Section 3.3.3).

In O. platytarsis, we have been able to detect inositol and ascorbic acid in CSG and vas deferens (aqueous and alkaline extracts), respectively. In the natantian prawn Penaeus indicus, inositol appears to be localized in the seminal vesicle; ascorbic acid has been detected in acidified extracts of the vas deferens (Muthuraman and K.G. Adiyodi, 1984). Inositol, a carbocyclic polyol, is present almost throughout the male reproductive system of Paratelphusa hydrodromous (Mathad, 1983). It is a constituent of the semen of several vertebrates, e.g. man, bull, ram and stallion (Mann, 1964 for reference). In boar semen, inositol content is quite high (532 mg/100 ml). The seminal vesicle secretion of boar being almost devoid of sodium chloride (Mann, 1953, 1954), inositol has been suggested

to play some role in maintaining the osmotic equilibrium of the seminal plasma. Inositol is also known to be important for the survival and growth of cultured mammalian cells in ways not clearly understood. Structural similarity between inositol and glucose as well as fructose is striking; Fischer (1945) thinks therefore that inositol in animal body may function as a sort of reserve-carbohydrate for hexoses. Inositol in CSG secretion may be used by O. platytarsis as a reserve for nourishment of the spermatozoa mainly within the spermatheca of the female to which the final semen is passed for storage following mating. Though some mixing of the primary semen with CSG secretion to form small quantities of final semen may also occur at the region where the main collecting duct from CSG enters the vas deferens, a large-scale mixing of the primary semen with CSG secretion occurs only during mating. Inositol is known to function also as a lipotropic and antiketogenic agent. It is not known whether inositol has this role in CSG.

Ascorbic acid, found in extracts of the vas deferens of O. platytarsis, has been also found in semen of many vertebrates including man. Among invertebrates, ascorbic acid has been detected in semen of Paratelphusa hydrodromous (Mathad, 1983), Scylla serrata (Uma and Subramoniam, 1981a) and Balanus balanus (Barne, 1962a). In Scylla serrata,

seminal plasma contains about 1.969 μg ascorbic acid, which is contributed mainly by the testis and proximal part of the vas deferens (Uma and Subramoniam, 1981a).

Our studies, however, show that the testis of O. platytarsis contains only lesser quantity of ascorbic acid than vas deferens.

The functional significance of ascorbic acid in semen remains enigmatic. Phillips et al. (1940) who investigated ascorbic acid levels in bull semen suggested that ascorbic acid might have some role in determining the fertility in bull in which it is the principal reducing agent in semen and vesicular secretion. Ascorbic acid, an important biologically active reductant (Chinoy et al., 1982), is suggested to play a key role to bring about the recovery of some histopathological conditions of the male reproductive system in rats (Chinoy and Chinoy, 1984). The required energy for a number of oxidoreductive reactions is found to be derived from monohydroascorbic acid (free unstable radical of ascorbic acid). Ascorbic acid can activate enzymes via the second messenger c-AMP (Chinoy, 1978). Pilyashenko-Novokhatnyi et al. (1983) have emphasized the importance of ascorbic acid in methane oxidation by methane-monooxygenase. Other suggested role of ascorbic acid are in protein, carbohydrate, lipid and mineral metabolism, and in the degradation of tyrosine

and phenylalanine. Ascorbic acid influences the motility and metabolism of human and rat spermatozoa (Chinoy, 1978). Chinoy and Seethalakshmi (1977) indicated the possible role of ascorbic acid in steroidogenesis in the male reproductive organs of the slug Laevicaulis alte. Seminal plasma of B. balanus has an ascorbic acid content of 21 µg/ml; here, ascorbic acid appears to serve as a protecting factor against the poisoning of -SH groups by thymol-reactive agents (Barnes and Finlayson, 1962). Addition of ascorbic acid to water induces mass copulation in B. balanus (Barnes, 1962a) by a mechanism not yet understood. Ascorbic acid has some influence on immune response of domestic animals (Corbel and Wood, 1975) and acts as an antitoxic agent (Sprince et al., 1975; Jagota et al., 1982; Sharma et al., 1982; Fodor et al., 1983).

The testis of O. platytarsis contains generally more chloroform-extractable lipid than either vas deferens or CSG_Aⁱⁿ both of which fluctuations in lipid levels are striking (Table 4.2). This observation of a high content of lipid in testis of O. platytarsis is in agreement with that of Mathad (1983) in the freshwater crab, P. hydrodromous. Testicular lipids of O. platytarsis include steroid compounds (including cholesterol) and their esters. In P. hydrodromous, cholesterol and its esters constitute the major part of testicular lipids (Mathad, 1983). In

bull testis, out of the total lipid content of about 16.8 mg/g, cholesterol accounted to about 1.9 mg/g (Melampy et al., 1955). In the freshwater teleost, Mystus M. vittatus, lipid and cholesterol content of the testis increases during spawning season (Pandey and Pandey, 1985). In mammals, the testicular tissue contains steroids such as testosterone, androstenedione and androstenol, all of which can be derived from cholesterol. It is not known whether cholesterol in O. platytarsis testis serves as a precursor for the synthesis of steroid hormones and also whether the testis contains any vertebrate-type steroid hormones, but in prawns and lobsters, Guary and Kanazawa (1973) and Kanazawa and Teshima (1971) have reported the possible conversion of cholesterol into sex hormones by the testis. Gilgan and Idler (1967) detected the presence of 17 β -hydroxysteroid dehydrogenase in lobster testis and it is suggested that androstenedione is converted to testosterone in this tissue. More recently, several vertebrate types of steroids have been identified by mass spectrometry in haemolymph of the crayfish Astacus leptodactylus: prenendone, 17 α -hydroxypregnenolone, testosterone, 11 β -hydroxytestosterone, 11-ketotestosterone, androstenedione, and dehydroepiandrosterone (De Loof, 1984 for review). These observations suggest that metabolic pathways of steroids in crustaceans may be similar to those of vertebrates.

In O. platytarsis, the vas deferens and CSG contain almost comparable quantities of lipids (an average of about 3.6%) (Table 4.2). These values of chloroform-extractable lipids are comparable to those of the vas deferens and seminal vesicle in Penaeus indicus (Muthuraman and K.G. Adiyodi, 1981). The lipid content of the semen of P. hydrodromous ranges from 7.20 to 20.10 mg/ml (Mathad, 1983), whereas that of the seminal plasma of B. balanus is only 1.7 mg/ml (Barnes and Blackstock, 1974). In the sea urchin Echinus esculentus, the seminal lipid is composed of neutral fat, free fatty acids, phospholipids and sterols (Cardin and Meara, 1953). In our histochemical studies (Chapter III) we could find droplets of neutral lipids in CSG. Prostatic fluid is the chief source of lipid in human semen in which it amounts to about 186 mg/100 ml (Scott, 1945). The fatty acids on oxidation via β -hydroxy- β -methyl glutaryl Co-A yield acetoactyl Co-A, which in turn gives rise to acetoacetate. The presence of acetone bodies in testis, vas deferens and CSG of O. platytarsis suggests that fatty acid degradation takes place in male reproductive system of this species.

Citric acid has been detected by us in vas deferens of O. platytarsis (Table 4.1). The vas deferens of Penaeus indicus also shows appreciable amounts of citric

acid (Muthuraman and K.G. Adiyodi, 1984). The physiological role of citric acid in O. platytarsis semen is obscure. The role of citric acid in vertebrate semen has been, however, studied in some detail. In human semen for example, the prostatic secretion is the main source of citric acid whereas in bull, ram and boar citric acid is contributed mainly by the seminal vesicle. In contrast, in P. indicus, the seminal vesicle contains no citric acid (Muthuraman and K.G. Adiyodi, 1984). Only small amounts of citric acid are present in semen of stallion, jackass and dog. The quantity of citric acid is considered as an indicator of androgenic stimulation (Gavella, 1983) and the quantity of citric acid increases with increase in sperm count (Sherief, 1983; Piironen and Hyvarinen, 1983). In vertebrate semen, citric acid has been suggested to be involved in activation of enzymes (Lundquist, 1947) and maintenance of osmotic equilibrium, and as a metal-binding factor (Mann, 1964).

The presence of some anionic (e.g. chlorides, oxalates, sulphates and phosphates) and cationic substances (e.g. calcium) in male reproductive system of O. platytarsis, is indicated by spot tests (Table 4.1). Vas deferens extracts of Penaeus indicus also reveal the presence of oxalates and calcium (Muthuraman and K.G. Adiyodi, 1984). Human prostatic secretion contains calcium (30 m.-equiv./L),

sodium (156 m.-equiv./L), potassium (30 m.-equiv./L), citrates (156 m.-equiv./L), chlorides (38 m.-equiv./L) and phosphates (1 m.-equiv./L) (Huggins et al., 1942). Several of these substances also are present in semen of most domestic animals. For example, sodium (181.25 ± 14.02 mg/100 ml), potassium (133.33 mg/100 ml), magnesium (6.22 ± 1.07 mg/100ml), calcium (5.65 ± 0.47 mg/100 ml) and chloride (278.89 ± 16.16 mg/100 ml) were detected in the pooled semen of ram (Daudu et al., 1983). Concentration of inorganic ions (like Ca, Mg, Zn) in seminal plasma reflect the functional capacity of accessory glands of the genital system (Adamopoulous and Deliyiannis, 1983; Arver, 1982). In seminal plasma of Balanus balanus, sodium occurs in greater concentration, while potassium and calcium are present in excess of their quantities in sea water. Chlorides are the most dominant inorganic constituent in seminal plasma of cirripedes (Barnes, 1962a). In boar semen, chloride is totally absent and the ionic equilibrium on the cation side is set up chiefly by potassium against citric acid, as the main anion. Phosphate has some favourable effects on motility and metabolism of the spermatozoa, whereas calcium ions have an adverse effect (Mann, 1964). Calcium has a role in stabilizing the surface composition (surface proteins) of spermatozoa (Vierula and Rajaniemi, 1983). In Cancer irroratus,

inorganic ions together with FAA form an important part of cell volume regulatory system (Moran and Pierce, 1984).

Phosphorus has been detected in testis, vas deferens and CSG of O. platytarsis. It is also present in semen of man and domestic animals.

The physiological role of phenol and phenolic compounds in semen of O. platytarsis is speculative. At least some crustaceans have phenolically tanned protein layers in their spermatophores (e.g. Penaeus trisulcatus: Malek and Bawab, 1971). It is not known whether the phenol present in O. platytarsis male reproductive system has any such role. Some workers (Soderhall, 1982; Pye, 1974) have suggested the possible role of phenol and phenoloxidase in preventing fungal and bacterial growth in animal systems. In reproductive system of O. platytarsis, O-nitrosophenol has not been found, but p-nitrosophenol is present.

Biochemical data presented in Table 4.2 show that in O. platytarsis the protein content of the hepatopancreas is far less than that of the vas deferens or CSG. This appears to be compensated in part by higher percentages of lipids in the hepatopancreas; O. platytarsis male

reproductive system contains comparatively only small quantities of lipids like the male reproductive system of Paratelphusa hydrodromous (Mathad, 1983). Interestingly, a comparable type of quantitative relationship between hepatopancreas and reproductive organs has been discovered to exist in female P. hydrodromous by Anilkumar (1980) in our laboratory. In this species, hepatopancreatic lipids are mobilized to meet the demands of vitellogenesis (R.G. Adiyodi and K.G. Adiyodi, 1970; Anilkumar, 1980). It is likely that the hepatopancreatic lipid reserves of male O. platytarsis may be utilized for reproduction and somatic growth, as in other decapods.

The hepatopancreatic lipid in O. platytarsis contains cholesterol. The cholesterol-positive lipid in Paratelphusa hydrodromous is suggested to be of dietary origin (R.G. Adiyodi and K.G. Adiyodi, 1971). In arthropods, the major functional steroid is cholesterol. The synthesis of moulting hormone from cholesterol has been reported in some crustaceans (Kater and Spaziani, 1971; Gagosian et al., 1974, Spindler et al., 1980 for review); it may be that cholesterol for biosynthesis of ecdysteroids by the Y-organ is supplied by the hepatopancreas. The concentrations of total lipid and cholesterol in hepatopancreas of the blue crab, Callinectes sapidus were higher than those of gonads. Moreover, in mature male C. sapidus, concentration

of both lipid and cholesterol in hepatopancreas decreased during the breeding period (Tasi et al., 1984). A comparable observation has been reported also in Scylla serrata (Farooqui and Nagabhushanam, 1983).

The FAA pool of the hepatopancreas of O. platytarsis is strikingly high and the fluctuations in its levels are ^{of} sweeping dimensions (Table 4.2). FAA seem to constitute the major source of hepatopancreatic nitrogen, as protein content of the hepatopancreas is comparatively small. Hepatopancreatic FAA may be formed from various sources, e.g. by direct absorption from the gut, by degradation of hepatopancreatic protein and by interconversion of other compounds. In insects, the fat body is the major site for the synthesis of haemolymph protein; in the rhinoceros beetle Oryctes rhinoceros, the profiles of FAA in fat body and haemolymph are comparable (Rajamma, 1975). In Paratelphusa hydrodromous there is some circumstantial evidence that hepatopancreatic FAA may be utilized for vitellogenic purposes (Anilkumar, 1980).

The oligosaccharide and polysaccharide contents of the hepatopancreas and vas deferens of O. platytarsis are comparable. The presence of reducing sugars (e.g. glucose), pentose, sucrose and glycogen are strongly indicated in the hepatopancreas of O. platytarsis. It has been concluded that the hepatopancreas is a storage

centre for carbohydrates in Paratelphusa hydrodromous (Anilkumar, 1980). The free sugars found in hepatopancreas of P. hydrodromous include glucose, sucrose and galactose (R.G. Adiyodi and K.G. Adiyodi, 1970). Organic reserves in hepatopancreas, reproductive organs and haemolymph of this species showed some correlation. The haemolymph of P. hydrodromous contains glucose, galactosamine, N-acetyl galactosamine and sucrose (Anilkumar, 1980). The free sugars present in haemolymph of O. platytarsis are maltotriose, maltose, fucose, glucose, galactose, raffinose and trehalose, and it is suggested that the carbohydrate reserves in haemolymph are available for more rapid mobilization than those of storage organs (Raja et al., 1976). In insects, fat body glycogen is suggested to provide the energy for the synthesis and transport of raw materials to developing oocytes (Wiens and Gilbert, 1967). In the isopod Lirceus brachyurus, the major part of glycogen reserve of the hepatopancreas is utilized as a metabolic substrate (Steeves, 1963).

Qualitative analysis shows that the hepatopancreas of O. platytarsis contains inositol, ascorbic acid, citric acid, phosphorus, phenols and phenolic compounds, various ions such as phosphates, chlorides, oxalates, sulphates and calcium. In P. hydrodromous, calcium is mainly concentrated as deposits in 'copper cells' of the hepatopancreas (R.G. Adiyodi, 1969). Amorphous calcium phosphate

was isolated from the hepatopancreas of blue crab, Callinectes sapidus (Belt's et al., 1975). The level of inorganic ions (e.g., calcium and magnesium) in the hepatopancreas of Oziotelphusa senex senex (= Paratelphusa hydrodromous) varies according to day length (Sreenivasulu Chetty et al., 1984). Similarly, photoperiod influences the level of ascorbic acid in hepatopancreas of the hermit crab, Clibanarius arethusa (Sarojini and Jahagirdar, 1980). According to Ramasubramaniam (1979), phenolic compounds have some role in pigmentation (yellow) and it is of interest to note that hepatopancreas of Q. platytarsis has a yellow colour during intermoult.

4.5. SUMMARY

1. Protein forms the most prominent organic constituent of the primary semen and of the extracts of the vas deferens (AVD+MVD-I) and CSG of Q. platytarsis, but the quantity of protein varies in different regions of the male reproductive system. Vas deferens and CSG contain albumin, proteoses, primary amines and tyrosyl residues.
2. Lipids and protein constitute the major organic components of Q. platytarsis testis. Testis has more free amino acids (FAA) than other parts of the male reproductive system.

3. In male reproductive system of O. platytarsis, testis alone showed the presence of phenylpyruvic acid and ammonia. O. platytarsis testis contained also glycogen, reducing sugars, glucose, oxalates, chlorides, steroids, sterols, phenols, acetone bodies and creatinine.
4. The vas deferens and CSG contained cystine/cysteine and tyrosine, but no tryptophan could be detected.
5. In CSG, oligosaccharide fraction is more dominant than polysaccharide fraction whereas in vas deferens the percentage of polysaccharides exceeds that of oligosaccharide fraction. Vas deferens and CSG contain reducing sugars, furfural-yielding substances and mucins.
6. Fructose, lactose and sucrose were not detected in the male reproductive system of O. platytarsis, but vas deferens and CSG contained glucose, pentose and glycogen.
7. CSG and vas deferens contain almost comparable quantities of lipids. Spot tests indicated the presence of acetone bodies in these tissues.
8. Neutral and alkaline extracts of vas deferens show the presence of ascorbic acid.

9. Appreciable amounts of calcium, oxalates and chlorides have been found in CSG and vas deferens of O. platytarsis. The presence of phosphates, inorganic sulphates, phenols, p-nitrosophenols and creatinine is indicated both in CSG and vas deferens. In male reproductive system, citric acid has been detected only in the vas deferens, and inositol only in the CSG.
10. Protein content of the hepatopaneas of male O. platytarsis is far less than that of the vas deferens or CSG, but lipid content (including cholesterol) of the hepatopaneas is more.
11. FAA pool of O. platytarsis hepatopaneas is strikingly high.
12. Oligosaccharide and polysaccharide contents of the hepatopaneas are comparable to those of the vas deferens. The presence of reducing sugars, sucrose, glucose, pentose and glycogen is strongly indicated in hepatopaneas of O. platytarsis.
13. Qualitative analysis shows that O. platytarsis hepatopaneas contains inositol, ascorbic acid, citric acid, phosphorus, phenols and phenolic compounds, phosphates, chlorides, oxalates, inorganic

sulphates and calcium, but substances such as O-nitrosophenol, galactose, lactose, tryptophan and tetrabase are absent.

Chapter V

Seasonal variation in male reproductive system of *Ocypode Platytarsis* Milne Edwards

M Sukumaran “Certain aspects of reproductive biology of the ghost crab, *Ocypode
Platyt Aris* with special reference to the male” Thesis. Department of Zoology,
University of Calicut, 1985

5.1. INTRODUCTION

Many marine crustaceans show a conspicuous annual periodicity in reproduction. Even in species described as continuous breeders, egg development and hatching do not occur during all seasons of the year (Subramoniam, 1977). Environmental factors such as photoperiod, temperature, salinity, abundance of food, etc. play an important role in breeding (Pillay and Nair, 1968; Cheung, 1969; Young, 1974; Aiken and Waddy, 1981). Preliminary studies by Pillay and Nair (1971) on some marine decapods have shown that the reproductive cycles in males and females are not exactly concurrent; similar results have been obtained on the freshwater crab, Paratelphusa hydrodromous in our laboratory (Mathad, 1983; R.G. Adiyodi, 1985). The breeding season in marine decapods extends for several months with distinct peak periods of gonadal activity. Though it is clear that cyclicity in reproductive physiology occurs in male decapods as well, our knowledge is fragmentary on biochemical changes that take place in male reproductive system of marine crabs during various seasons. This prompted us to study the seasonal variations in testis, vas deferens, accessory sex gland and androgenic gland of O. platytarsis the results of which are presented and discussed in this chapter.

5.2. MATERIALS AND METHODS

Mature male Ocypode platytarsis of average size (carapace width 4.5-6.0 cm) were collected every month from Calicut beach and were sacrificed immediately on arrival in the laboratory. A minimum of five crabs were used for each type of investigation. Histological (Section 3.2.2), histochemical (Section 3.2.3) and biochemical (Section 4.2.2) studies on different parts of the male reproductive system were conducted as described previously. Special care was taken to include all parts of the testis in studies on spermatogenic activity. Paraffin sections (5 μ m thick) of the androgenic glands were stained also with Gomori's chrome haematoxylin-phloxin.

The 'vas deferens factor' (Vdf) was calculated during different months after Aiken and Waddy (1981) using the formula:

$$\frac{\text{Weight of the vas deferens (AVD+MVD-I) in mg}}{(\text{Carapace width})^3 \text{ in cm}} \times 100 = \text{Vdf}$$

The 'accessory sex gland factor' (Glf) was calculated likewise, using the formula:

$$\frac{\text{Weight of the coral-shaped accessory sex gland (CSG) in mg}}{(\text{Carapace width})^3 \text{ in cm}} \times 100 = \text{Glf}$$

October through December, our collections included crabs in postmoult, intermoult and premoult, but only intermoult crabs were included in our study.

5.3. RESULTS

5.3.1. Seasonal Variations in Spermatogenesis

The process of spermatogenesis in O. platytarsis was studied light microscopically using sections of testis stained with Heidenhain's haematoxylin-alcoholic eosin. Various stages of spermatogenesis could be identified in the same animal. Spermatogonia (about 5.0-7.5 μ m in diameter) are rounded or oval with a prominent nucleus. Primary spermatogonia are distributed in acini toward the periphery and secondary spermatogonia toward the interior. Nuclear basophilia of the secondary spermatogonia is more intense than that of the primary spermatogonia. There are certain elongated, deeply basophilic mesoderm cells distributed in inter-acinar spaces and toward the boundary of testicular acini of O. platytarsis. However, individual spermatogonia are seldom encircled by mesoderm cells. Primary spermatocytes are almost of the same size as secondary spermatogonia, but could be distinguished from them by their characteristic pattern of chromatin clumping. It should be mentioned that in O. platytarsis primary spermatocytes in various stages of first meiotic division

have been observed by us only in a few instances. The secondary spermatocytes of O. platytarsis exist in two distinct phases. In the first phase (ScR), they are spherical or disc shaped (4.38-5.0 μm in diameter) (Figs. 5.1 and 5.2). The chromatin material is highly basophilic and has a homogeneous appearance. In the second phase (ScD), they enter meiotic division-II. ScD (Figs. 5.1 and 5.3) measure about 5.0-6.25 μm in diameter. The pattern of chromatin distribution representing various stages of cell division (such as in metaphase, telophase, etc.) is characteristic of ScD. At least in a few acini, a gradual transformation of ScR through ScD into spermatid could be observed; ScR is found toward one end of the acinus, ScD in the middle, and spermatid toward the other end of the acinus. Thus, it seems that ScR represents a 'resting' phase of the spermatocyte, before entering meiotic division-II. Acini containing ScR are common in O. platytarsis testis.

Spermatids measure about 2.5 μm in diameter. The spermatids of O. platytarsis in their early stages of development (SpE) (Figs. 5.1 and 5.4) are characterized by condensed chromatin which is almost basophilic and has irregular contours. As spermiogenesis advances, the pattern of chromatin distribution changes and the condensed chromatin becomes more spherical (Fig. 5.5); there is a

decline in basophilia and a central acidophilic region develops. Such spermatids (S_pL) are almost comparable to spermatozoa, but differ from them in the pattern of distribution of chromatin. The disc-shaped spermatozoa have a diameter of 2.5-3.0 μ m.

In sections of testis of O. platytarsis stained with mercury bromophenol blue, the spermatocytes stain more intensely than either spermatogonia or spermatids, indicating high concentration of proteins in spermatocytes. Spermatids are intensely positive to PAS, whereas spermatogonia and spermatocytes are only moderately so.

In O. platytarsis, all the germ cells in a given acinus are generally in the same stage of spermatogenesis. In order to clarify the relative abundance of various spermatogenic stages in testis, the number of acini containing each stage was counted in serial sections (stained with Heidenhain's haematoxylin-alcoholic eosin) at random so as to include all parts of the testis. The percentage of acini containing each stage was calculated in terms of the total number of acini counted (Table 5.1). Very rarely, spermatocytes plus spermatids or spermatozoa have been seen in the same acinus (in such instances, the number of each type of stage was calculated in fractions).

The results showed that the percentage of testicular

Table 5.1

Spermatogenesis in O. platytarsis: Seasonal variations in the percentage of germ cells

Month	Spermato- gonia	Primary Spermato- cytes	ScR	ScD	SpE	SpD	Spermatozoa
January- February	1.49	1.84	2.43	47.99	26.78	10.24	8.66
March- April	0.41	0.35	1.15	14.00	48.31	24.94	10.32
June-July	0.74	0.45	2.78	29.78	54.90	5.63	4.85
September- December	3.79	0	4.47	26.59	53.43	6.83	2.31

acini containing different stages of spermatogenesis in O. platytarsis varied considerably in different months of the year. Acini containing spermatogonia or primary spermatocytes were, in general, much less during various seasons. However, spermatogonia were more frequently observed during September/October. Primary spermatocytes undergoing meiotic division-I were common during January/February. It was not possible to observe even a single stage of first meiotic division in spermatocytes during September/October. The number of acini containing ScR reached peak values during September/October and was at its lowest during March/April. Both secondary spermatocytes undergoing division (ScD) and spermatids were abundant in a majority of the acini almost throughout the sampling periods. About 47.99% of the total acini were in stage ScD during January/February, but only 14.00% in March/April. The percentage of SpE was maximum (55.89%) during June/July, and minimum (26.78%) during January/February. By March/April, a majority of the acini contained spermatids in various stages of spermiogenesis and also mature spermatozoa, whereas in September/October the acini containing spermatozoa were rare.

5.3.2. Seasonal Variations in Androgenic Glands

The androgenic glands of O. platytarsis consist of a number of parallel wavy strands. The volume of the

glands was found to be more during January-April than during the rest of the year. Histological observations revealed that the cells of androgenic glands were larger during January-February (Fig. 5.6) they had a relatively dense and homogeneous cytoplasm which contained few vacuoles. The nuclei were large with prominent nucleoli; the shape varied from spherical to oval. Multinucleolated condition was common. The nucleus had the characteristic chromatin clumping. Conspicuous eosinophilic spaces could be seen between the cell groups of various strands. However, even during this active phase, there were no signs of cytoplasmic basophilia (Heidenhain's haematoxylin-alcoholic eosin staining) or cytoplasmic droplets (Gomori's chrome-haematoxylin-phloxin staining) in androgenic gland cells of O. platytarsis. Pycnotic nuclei were far less numerous during this season.

During June-July, the volume of the androgenic gland was less than during January-February. The cytoplasm was more compact during this period and the cells were smaller in size. (Fig. 5.7). In some cases vacuoles were present. In nuclear morphology the androgenic glands of June-July crabs were comparable to those of February-March crabs, but pycnotic nuclei were more common.

During September-October, the androgenic gland of O. platytarsis was found to shrink further in size and to

consist of only a few cords of cells which were closely associated with each other. The cells were small and the cytoplasm rather scarce. The boundaries of gland cells were far from clear at the light microscopic level. The nuclei were indistinct and were usually pycnotic. In this inactive state, the androgenic gland as a whole stained only lightly, and the affinity of gland cells to basophilic stains was found to be remarkably low (Fig.5.8).

5.3.3. Seasonal Fluctuations in Vas Deferens and CSG of O. platytarsis.

In O. platytarsis, the sperm duct was found to be filled with semen during nearly all seasons. The histological profiles of vas deferens and CSG (see Section 3.3.3) changed little through various months. However, the cells of AVD-I and AVD-II showed a slightly more intense cytoplasmic basophilia during February-March than during other seasons. The nuclei of epithelial cells of AVD-I, AVD-II and CSG became more prominent and CSG epithelium showed signs of greater activity during this period.

Tables 5.2 to 5.11 show variations in major biochemical components of the vas deferens and CSG of O. platytarsis during different sampling periods. A comparison between the 'vas deferens factor' (Vdf) and protein content of

Fig. 5.1 Section of testis of O. platytarsis showing spermatocytes (ScR, ScD) and spermatids (SpE) (see text for details).
Formol-saline, Heidenhain's haematoxylin-alcoholic eosin X 250

Fig. 5.2 Section through testicular acinus of O. platytarsis to show spermatocytes (ScR).
Formol-saline, Heidenhain's haematoxylin-alcoholic eosin X 300

Fig. 5.3 Section through testicular acinus of O. platytarsis. Note ^{the} spermatocytes (ScD).
Formol-saline, Heidenhain's haematoxylin-alcoholic eosin X 330

Fig. 5.4 Section through testicular acinus of O. platytarsis to show spermatids (SpE).
Formol-saline, Heidenhain's haematoxylin-alcoholic eosin X 480

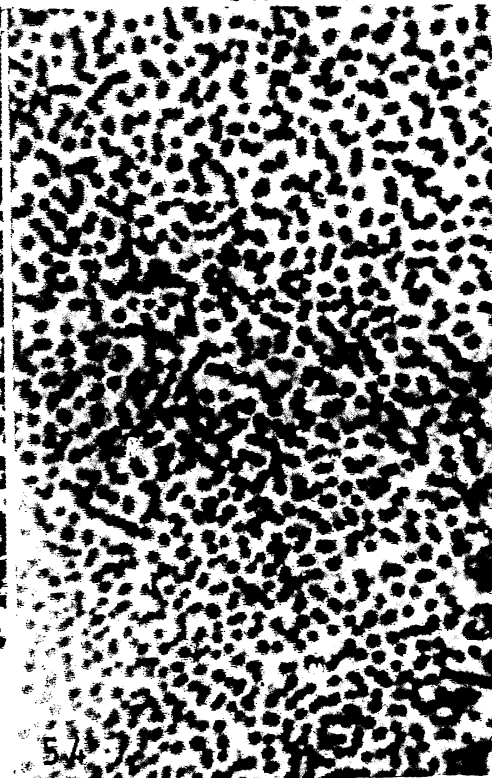
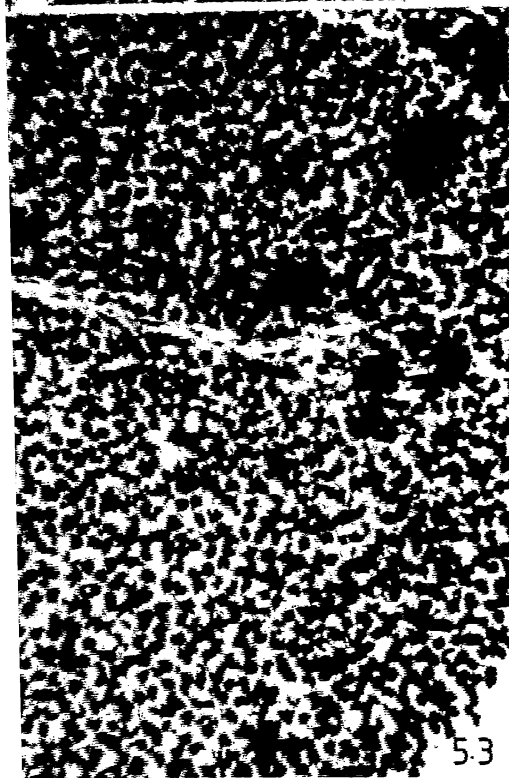
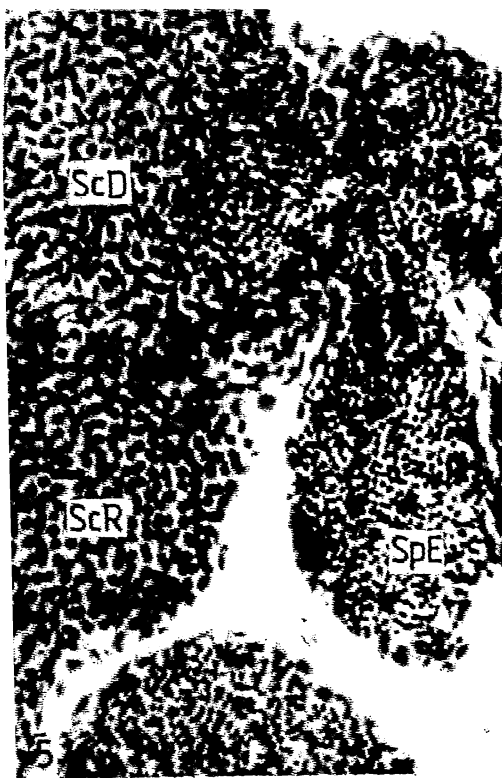
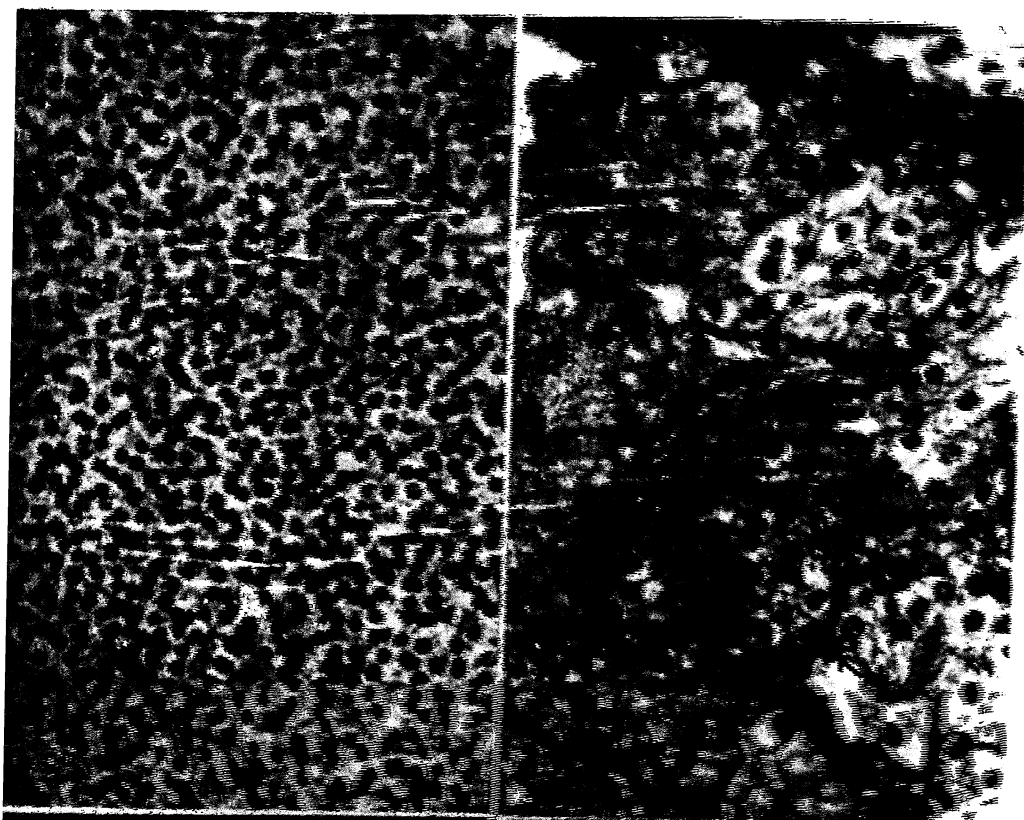


Fig. 5.5 Section through testicular acinus of O. platytarsis to show spermatids (SpL).
Formol-saline, Heidenhain's haematoxylin-alcoholic eosin X 480

Fig. 5.6 Cross section through androgenic gland of O. platytarsis during January.
Note the large size of the gland cells themselves and of their nucleus (N).
Bouin's, Gomori's chrome - haematoxylin-phloxin X 380

Fig. 5.7 Cross section through androgenic gland of O. platytarsis during June. The gland cells are now smaller in size and undergoing degeneration.
N - nucleus
Bouin's, Gomori's chrome - haematoxylin-phloxin X 380

Fig. 5.8 Cross section through androgenic gland of O. platytarsis during September. The gland is reduced to a few cords of cells, cytoplasm is scarce and nucleus indistinct or pycnotic (N)
Formol-saline, Heidenhain's haematoxylin-alcoholic eosin X 400



FLUCTUATIONS IN PROTEIN CONTENT OF THE VAS DEFERENS
AND ITS RELATIONSHIP WITH THE VAS DEFERENS FACTOR (Vdf)
IN OCYPODE PLATYTARSIS AS REVEALED FROM A YEAR-ROUND
ANALYSIS

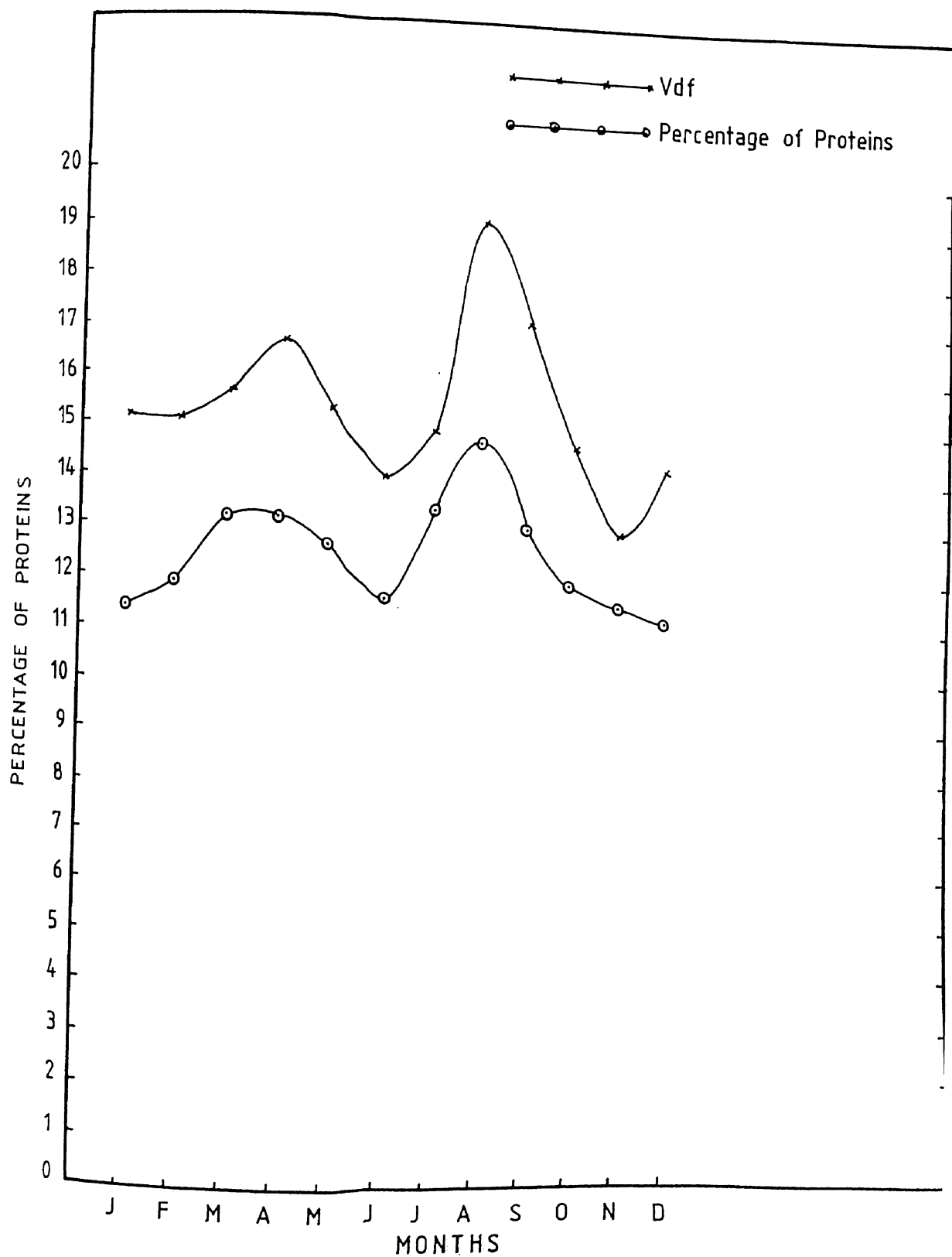


Fig. 5.9

FLUCTUATIONS IN PROTEIN CONTENT OF THE CSG AND ITS
RELATIONSHIP WITH THE CSG FACTOR(Glf) IN OCYPODE
PLATYTARSIS AS REVEALED FROM A YEAR-ROUND ANALYSIS

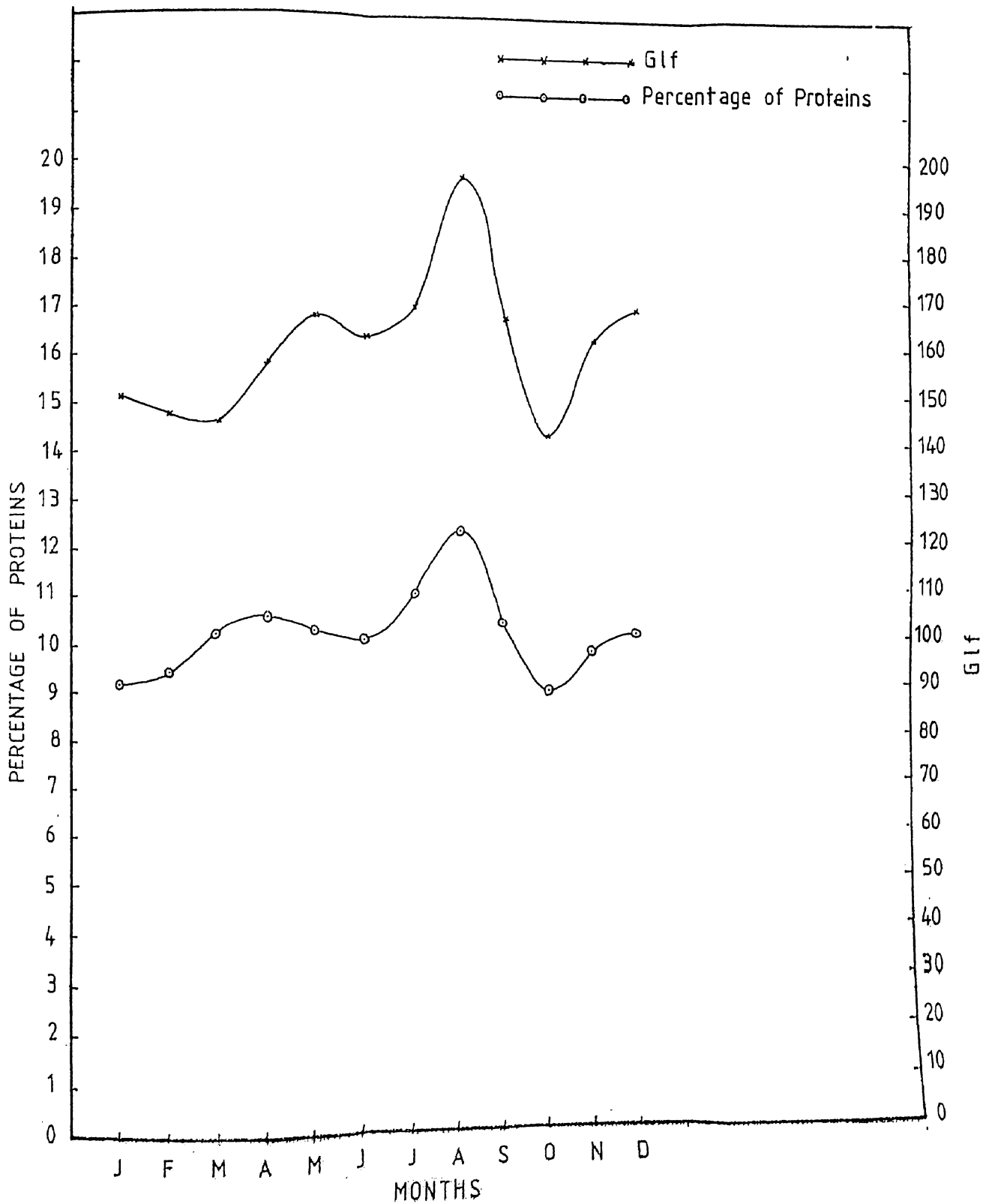


Fig. 5.10

Table 5.2

Fluctuations in protein content of the CSG of O. platytarsis during various seasons (quantity expressed as mg/100 mg ($\bar{X} \pm$ SD) of the wet weight of the tissue).

(n=5)

(n=3)

March/April I	June/July II	August III	October/November IV	December/January V
10.67 ± 0.897	10.338 ± 1.087	12.306 ± 0.462	9.136 ± 0.181	9.899 ± 0.136
Level of significance:				
I = II	t = 0.527	NS		
I < III	t = 3.62	P < 0.01	II > IV	t = 2.439 P < 0.05
I > IV	t = 3.75	P < 0.01	II = V	t = 0.895 NS
I = V	t = 1.91	NS	III > IV	t = 14.24 P < 0.001
II < III	t = 3.75	P < 0.01	III > V	t = 11.14 P < 0.001
			IV < V	t = 7.52 P < 0.001

NS = not significant

NS = not significant

Table 5.3.

Fluctuations in free amino acid (FAA) content of CSG of O. platytarsis during various seasons (quantity expressed as mg/100 mg ($\bar{X} \pm$ SD) of the wet weight of the tissue).

(n=5)

March/April I		June/July II	August III	October/November IV	December/January V
0.339 ± 0.033		0.109 ± 0.069	0.117 ± 0.091	0.181 ± 0.119	0.068 ± 0.02
Level of significance:					
I	> II	t = 6.752	P < 0.001	II =	IV = 1.151
I	> III	t = 5.08	P < 0.001	II =	V = 1.323
I	> IV	t = 2.86	P < 0.05	III =	IV = 0.951
I	> V	t = 17.356	P < 0.001	III =	V = 1.18
II	= III	t = 0.156	NS	IV =	V = 2.089
NS = not significant					

Table 5.4

Fluctuations in oligosaccharide fraction of the CSG of O. platytarsis during various seasons (quantity expressed as mg/100 mg ($\bar{X} \pm$ SD) of the wet weight of the tissue).

(n=5)

March/April I		June/July II		August III		October/November IV		December/January V			
0.444 $\pm$ 0.035		0.261 $\pm$ 0.036		0.902 $\pm$ 0.343		0.768 $\pm$ 0.083		0.559 $\pm$ 0.049			
<u>Level of significance:</u>											
I	> II	t =	8.09	P <	0.001	II	< IV	t =	12.582	P <	0.001
I	< III	t =	2.98	P <	0.02	II	< V	t =	10.89	P <	0.001
I	< IV	t =	8.07	P <	0.001	III	= IV	t =	0.848	NS	
I	< V	t =	4.25	P <	0.005	III	= V	t =	2.22	NS	
II	< III	t =	4.16	P <	0.005	IV	> V	t =	4.85	P <	0.002

NS = not significant

Table 5.5

Fluctuations in polysaccharides of CSG of O. platytarsis during various seasons (quantity expressed as mg/100 mg ($\bar{X} \pm SD$) of the wet weight of the tissue).

(n=5)

March/April I	June/July II	August III	October/November IV	December/January V
0.128 $\pm$ 0.003	0.119 $\pm$ 0.029	0.100 $\pm$ 0.068	0.177 $\pm$ 0.011	0.215 $\pm$ 0.102
<u>Level of significance:</u>				
I = II	t = 0.791	NS	II = IV	t = 0.159
I = III	t = 0.940	NS	II = V	t = 2.011
I > IV	t = 2.432	P < 0.05	III = IV	t = 0.539
I = V	t = 1.864	NS	III = V	t = 2.100
II = III	t = 0.572	NS	IV = V	t = 2.125
NS = not significant				

Table 5.6

Fluctuations in lipid content of the CSG of O. platytarsis during various seasons (quantity expressed as mg/100 mg ($\bar{X} \pm \text{SD}$) of the wet weight of the tissue).

(n=5)

March/April I	June/July II	August III	October/November IV	December/January V
3.468 $\pm$ 1.43	4.518 $\pm$ 1.26	4.44 $\pm$ 1.30	4.244 $\pm$ 1.08	2.52 $\pm$ 0.717
<u>Level of significance:</u>				
I = II	t = 1.23	II	t = 0.369	NS
I = III	t = 1.13	II	t = 3.08	P < 0.02
I = IV	t = 0.969	III	t = 0.259	NS
I = V	t = 1.33	III	t = 2.89	P < 0.05
II = III	t = 0.96	IV	t = 2.98	P < 0.02

NS - not significant

Table 5.7

Fluctuations in protein content of the vas deferens of O. platytarsis during various seasons quantity expressed as mg/100 mg ($\bar{X} \pm$ SD) of the wet weight of the tissue).

(n=5)

March/April I		June/July II	August III	October/November IV	December/January V
13.002 ± 1.00		12.481 ± 1.321	14.77 ± 2.262	11.936 ± 0.556	11.372 ± 0.137
Level of significance:					
I =	II	t =	0.703	NS	
I =	III	t =	1.599	NS	
I =	IV	t =	2.083	NS	
I >	V	t =	3.613	P < 0.01	P < 0.05
I =	III	t =	1.95	NS	P < 0.01
				t =	2.206 NS

able 5.8

luctuations in free amino acid content of the vas deferens of O. platytarsis during various seasons (quantity expressed as mg/100 mg ($\bar{X} \pm SD$) of the wet weight of the tissue).

(n=5)

arch/April I	June/July II	August III	October/November IV	December/January V
329 $\pm$ 0.037	0.077 $\pm$ 0.016	0.249 $\pm$ 0.012	0.206 $\pm$ 0.032	0.148 $\pm$ 0.066
vel of significance:				
> II	t = 14.186	P < 0.001	II <	IV
> III	t = 4.59	P < 0.002	II <	V
> IV	t = 5.655	P < 0.001	III <	IV
> V	t = 5.303	P < 0.001	III >	V
< III	t = 19.19	P < 0.001	IV =	V
- not significant				
			t = 8.020	P < 0.001
			t = 2.340	P < 0.05
			t = 2.81	P < 0.05
			t = 3.36	P < 0.01
			t = 1.717	NS

Table 5.9

Fluctuations in oligosaccharide fraction of the vas deferens of O. platytarsis during various seasons (quantity expressed as mg/100 mg ($\bar{X} \pm SD$) of the wet weight of the tissue).

		(n=5)			
March/April I		June/July II	August III	October/November IV	December/January V
0.379 ± 0.052		0.573 ± 0.019	1.107 ± 0.177	0.264 ± 0.061	0.365 ± 0.093
<u>Level of significance:</u>					
I < II	t =	7.84	P < 0.001	II > IV	t = 10.764 P < 0.001
I < III	t =	8.83	P < 0.001	II > V	t = 4.346 P < 0.002
I < IV	t =	3.21	P < 0.02	III > IV	t = 10.08 P < 0.001
I = V	t =	0.231	NS	III > V	t = 8.26 P < 0.001
II < III	t =	6.71	P < 0.001	IV = V	t = 2.105 NS

NS - not significant

Fluctuations in polysaccharide content of the vas deferens of O. platytarsis during various seasons (quantity expressed as mg/100 mg ($\bar{X} \pm$ SD) of the wet weight of the tissue).

March/April I	June/July II	August III	October/November IV	December/January V
0.471 ± 0.246	0.380 ± 0.110	0.261 ± 0.095	0.351 ± 0.075	0.916 ± 0.228
<u>Level of significance:</u>				
I = II	t = 0.753	NS		
I = III	t = 0.178	NS		
I = IV	t = 1.049	NS		
I < V	t = 2.964	P < 0.02		
II = III	t = 1.83	NS		
		II = IV	t = 0.502	NS
		II < V	t = 4.726	P < 0.002
		III = IV	t = 1.66	NS
		III < V	t = 5.93	P < 0.001
		IV < V	t = 5.261	P < 0.001

Fluctuations in lipid content of the vas deferens of O. platytarsis during various seasons (quantity expressed as mg/100 mg ($\bar{X} \pm \text{SD}$) of the wet weight of the tissue).

March/April I	June/July II	August III	October/November IV	December/January V
2.67 ± 0.913	4.02 ± 0.838	3.40 ± 1.26	3.87 ± 1.15	3.40 ± 1.82
<u>Level of significance:</u>				
I < II	t = 2.45	P < 0.05	IV =	t = 0.229
I = III	t = 1.05	NS	II =	t = 0.692
I = IV	t = 1.83	NS	III =	t = 0.617
I = V	t = 0.802	NS	III =	t = 0
II = III	t = 0.917	NS	IV =	t = 0.493

the vas deferens, and between the 'gland factor' (Glf) and protein content of the CSG is given in Figs. 5.9 and 5.10 respectively.

5.4 DISCUSSION

Our observations have shown that the activity of the male reproductive system of mature male O. platytarsis fluctuates within small limits during different seasons. The vas deferens and accessory sex gland (CSG) tubules of this species have been found filled with secretions throughout the sampling periods, indicating the preparedness in principle of male O. platytarsis to mate throughout the year. Two other species of tropical crabs investigated in our laboratory also show this phenomenon (e.g. Paratelphusa hydrodromous: Mathad, 1983; Gecarcinus stenlops: Samuel, 1984). But in natantians (Penaeus indicus: Muthuraman and K.G. Adiyodi, 1980; Macrobrachium idella: Sreekumar and R.G. Adiyodi, 1981) semen production is low during the summer, which is generally a period of dull male reproductive activity. The quantity of semen contained in vas deferens and the nature of spermatogenic activity (Young, 1974) are two parameters by which reproductive potential of male crustaceans can be judged. Even in crabs containing copious amounts of semen in vas deferens (e.g. Paratelphusa hydrodromous) during all times of the

year, changes do occur in biochemical components of the vas deferens and semen in tune with the reproductively active and inactive seasons of the species (Mathad, 1993).

In O. platytarsis, the relative abundance of different stages in spermatogenesis shows fluctuations in relation to different seasons (Table 5.1). Throughout the sampling periods, secondary spermatocytes and spermatids dominated in all the lots. Germ cells in certain stages of development (e.g. spermatogonia and primary spermatocytes) were only poorly represented in a majority of the samples. The present study was restricted to crabs in intermoult stages of their moulting cycle: it is not unlikely that those stages of spermatogenesis which are rare in the present sampling may dominate in other phases of the moulting cycle (i.e. in premoult or postmoult) of this species. Moult-related changes in spermatogenesis have in fact been described in the shrimp, Macrobrachium idella (Sreekumar and R.G. Adiyodi, 1981). In M. idella, the acini are uniformly filled with spermatozoa during intermoult whereas in early postmoult contain mostly only spermatids. During early premoult (D_2 - D_3), the acini are dominated by secondary spermatogonia and primary spermatocytes, whereas in late premoult (D_4) primary and secondary spermatocytes are present almost in equal proportions in most acini. During late postmoult, the testicular acini show only spermatids

and spermatozoa (Sreekumar and R.G. Adiyodi, 1981). In the present investigation, the paucity of the primary spermatocytes (undergoing meiotic division-I) in many instances could be alternatively related to the very short duration of this stage as is apparently the case in mysids (cf. Kasaoka, 1974).

In testis of O. platytarsis, the germ cell population in a given acinus, are generally in the same stage of spermatogenesis. Such synchrony in development of male gametes has been observed also in the freshwater crabs P. hydrodromous (Mathad, 1983), Potamonkooloonese (Joshi and Khanna, 1982) and the brine shrimp Artemia (Wolfe, 1971). However, a given testicular acini of M. idella (Sreekumar and R.G. Adiyodi, 1983) and P. indicus (Muthuraman and K.G. Adiyodi, 1980) normally contain cells in more than one stage of spermatogenesis.

Spermatogonia of O. platytarsis are comparable in cytomorphology to those of P. hydrodromous, studied by Mathad (1983) in our laboratory. In both species, individual spermatogonia are not encircled by mesoderm cells. In Macrobrachium idella (Sreekumar and R.G. Adiyodi, 1983), the primary spermatogonia are approximately 10 μm in diameter, whereas the secondary spermatogonia are slightly smaller (6.25 to 7.5 μm). In Artemia, the spermatogonia are about 10 μm in diameter and are seen

in clusters throughout the length of the testis (Wolfe, 1971). The spermatogonia of M. idella, unlike those of O. platytarsis, show a more intense reaction to mercury bromophenol blue than spermatids or spermatocytes (Sreekumar and R.G. Adiyodi, 1983; Sreekumar, 1985). In many crustaceans, electronmicroscopic observations have revealed that the cytoplasm of primary spermatogonia contains mitochondria with transverse cristae, some dictyosomes, free ribosomes, a chromatoid body and a dense material called "clouds" associated with the mitochondria. The cytoplasm of secondary spermatogonia is richer in RNA and the nucleus has an apparently denser chromatin (Pochon-Masson, 1983).

Various steps ultimately leading to the production of spermatozoa proceed in animals either uninterrupted once started, or the transformation of some stages to the next ones may be influenced by specific internal or external factors. In the crayfish Pontastacus leptodactylus leptodactylus, for example, the conversion of primary spermatogonia is determined by the presence or absence of the mesodermal tissue. Moreover, in this species, the initiation of meiosis is said to be influenced by a male factor originating from the brain. The effect of this male factor on secondary spermatogonia is more pronounced when there is increased synthesis of RNA (in

secondary spermatogonia) which in turn is suggested to be under the control of the androgenic hormone (Amato and Payen, 1978). In O. platytarsis, the secondary spermatocytes exist in two stages, viz. the 'resting' stage (ScR) and the other undergoing division (ScD). Incidentally, two stages have been also seen in primary spermatocytes of Macrobrachium idella: one in meiotic division-I and the other in 'growth phase'; after the first meiotic division (Sreekumar and R.G. Adiyodi, 1983; Sreekumar, 1985). O. platytarsis spermatids have been also observed in the early and late stages of spermiogenesis. Perhaps different phases in spermatidogenesis and spermiogenesis in O. platytarsis do not quickly follow each other. In the crab Pinnixia sp., spermatids in early stages have a high nucleo-cytoplasmic ratio. Gradually, the chromatin becomes peripherally distributed within the nucleus. In late spermatids, the nucleus comes to occupy the entire cellular volume. In between developing spermatids, interstitial cells are present (Reger, 1970). During the maturation of spermatids in Neomysis, the chromatin material is clumped around the periphery of the nucleus, which later attains a 'tear-drop' shape. The central portion of the nucleus contains nucleoplasm and a few nucleoli (Kasaoka, 1974).

O. platytarsis spermatocytes show an intensely positive reaction to mercury bromophenol blue, suggesting the presence of a high concentration of proteins. This is not surprising, for spermatocytes which are metabolically very active have appreciable ability to synthesize RNA and proteins in crustaceans (Brasiello, 1971); in fact, the pachytene stage shows the maximum RNA content (Pochon-Masson, 1983). In crayfish, the secondary spermatocytes have much agranular reticulum and well organized masses of annulate lamellae (Anderson and Ellis, 1967).

The quantity of PAS-positive substances in O. platytarsis spermatids is relatively more than in spermatogonia or spermatocytes. In the freshwater crab, Paratelphusa hydrodromous, the spermatozoa are more strongly positive to PAS than are spermatogonia (Mathad, 1983). In Artemia, there is a decline in glycogen content of spermatogonia as maturity advances, but the germ cells at all stages of spermatogenesis contain glycogen and alkaline and acid phosphatases (Wolfe, 1971).

In the present study, major changes in spermatogenesis of O. platytarsis during different seasons have been found to pertain chiefly to populations of secondary spermatocytes and spermatids. Percentual composition of ScD, SpE and SpL

shows much variation during different sampling seasons. Various stages of spermatogenesis are more common in testis, March through July; in fact, many of the testicular acini of O. platytarsis are occupied by spermatids and spermatozoa during March/April. In summary, in O. platytarsis, though the process of spermatogenesis occurs during all seasons, the progress of spermatogenesis and production of spermatozoa are not uniform throughout the sampling periods. Output of spermatozoa and other spermatogenic processes appear to be at their highest during the first half of the year. In the freshwater crab Potamon koolooense, the spermatogenesis is seasonal; all the testicular acini do not mature simultaneously. Spermatogenesis starts during January-February and progresses slowly during March. Spermatogenic processes are maximum during April/May; spermiation occurs during May/June. Potamon testis is inactive in December (Joshi and Khanna, 1982). In Uca pugnax, there are two waves of production of spermatozoa during summer. During the non-breeding seasons, spermatogenesis and mating behaviour do not occur, but large numbers of mature spermatozoa and spermatophores are nevertheless seen in the sperm duct (Young, 1974). Interestingly, the annual spermatogenic cycle of the crayfish, Pontastacus leptodactylus leptodactylus is restrained by two "blocking" stages, the first one during winter is effected by the mesoderm cells and the second which lasts till the end of

summer, restrains the initiation of meiosis (Amato and Payen, 1978).

A casual relationship between androgenic gland hormone and the spermatogenic process has been suggested in crustaceans (Section 1.9.2. for details) and our observations seem to suggest this may be the case in also O. platytarsis. In O. platytarsis, during January–February, the androgenic glands are large in size and the cytoplasmic content of the cells is high. The nuclei of androgenic gland cells during this period are large with prominent nucleoli and show a strong affinity to basophilic stains — cytological features suggestive of an active gland. However, even during the 'active' phase it was not possible to detect any cytoplasmic granules in androgenic gland cells of O. platytarsis; this observation agrees with the findings of Thampy and John (1970) on androgenic glands of the same species. According to Thampy and John (1970), the androgenic glands are cylindrical and roughly circular in young crabs; as the animal grows, the width of the gland increases unevenly so that the gland appears branched and acquires a morphology unique among the brachyrrhynchan crabs. Androgenic gland cells of O. platytarsis are considered to be monophasic and holocrine (Thampy and John, 1970).

Though the chemical nature of secretion of the androgenic gland is disputed (see Section 1.9.2.) most workers (e.g. Charniaux-Cotton, 1960; Charniaux-Cotton and Kleinholz, 1963; Tcholakian and Reichard, 1964; Thampy and John, 1973; Radu and Cracium, 1976) agree that the gland secretes in a holocrine manner. This assumption is based on the presence of degenerating cells in the gland during periods when the gland is considered to be functional. The present study reveals that during June/July the androgenic gland cells of O. platytarsis are degenerated and reduced in size and their nuclei become pyconotic. However, from January through March, the cells are filled with cytoplasm; the nuclei are prominent, and degenerated cells are scarce. If androgenic gland cells of O. platytarsis also secrete in a holocrine fashion, it is quite possible that the titre of the androgenic hormone in the animal would be maximum towards the middle of the year, since degenerated cells are more common in androgenic gland from May to July. The large, active cells seen in androgenic gland towards the beginning of the year may represent the initial proliferative phase. During September/October the androgenic gland becomes smaller and the cells show less affinity for routine histological stains. All these indicate that the androgenic gland of O. platytarsis is in a comparatively inactive phase during September/October.

Investigations on other species of crustaceans have shown that changes in activity of androgenic glands proceed on similar lines as those in O. platytarsis. For example, in the shrimp Macrobrachium idae (Thampy and John, 1973), androgenic gland presents three types of histologically different pictures. The first type consists of the young tissue in which the centrally nucleated cells are filled with dense, homogeneous, and deeply staining cytoplasm; the cell boundaries are not distinct. In the second type, the cells contain vesicular cytoplasm with vacuoles. The spherical nuclei have the same inclusions as in the first type. The third type consists of degenerating tissue. Cell boundaries are not^{at} all distinct at the light microscopic level and even the individual cords are rarely discernible. The disintegrated cytoplasm shows no affinity for usual histological stains. The nuclei are reduced in size and the nucleoplasm shows little stainability. In animals with hypertrophied sex characters, the third type of androgenic gland is the most common, though the first and second types are also present. On the contrary, in animals without hypertrophied sex characters, major part of the gland is formed of the second type, the first and third type being comparatively rare. The extent of degenerating tissue is considerably more in animals with long chilepeds. Thampy and John (1973) therefore conclude

that androgenic hormone is liberated in animals with hypertrophied sex-characters.

In Orconectes nais, a more complex relationship exists between androgenic gland and activity of the male reproductive system (Carpenter and deRoos, 1970). In this crayfish, the moulting from sexually inactive Form-II to sexually active Form-I occurs in late August and early September. Prior to this moult, the androgenic gland attains its maximum size. Large hypertrophied cells with vesicular cytoplasm and areas of cellular degeneration are characteristic of the histology of active androgenic glands. During spring, the reverse type of moulting (i.e., from Form-I to Form-II) takes place, when the androgenic gland is small and is composed of only small cells. The androgenic gland is, much regressed during winter, whereas during summer there is a considerable increase in its size. Testicular maturation and spermatogenesis take place at the onset of summer. Though spermatozoa were present in vas deferens of O. nais during August, there were no signs of spermatogenesis. Testicular activity thus precedes the proliferation of androgenic gland in this species. Carpenter and deRoos (1970) maintain that prior to and during its proliferation stage the androgenic gland of O. nais is sufficiently active to stimulate testicular maturation and spermatogenesis. However, the increased

level of androgenic hormone, necessary for the moult from Form-II to Form I, becomes available only when there is an increase in the number of hypertrophied cells and when there are more extensive areas of cellular degeneration, provided the presence of areas of hypertrophy and degeneration is a sign of holocrine secretion.

In the protandric shrimp Pandalus platyceros, the androgenic glands grow in size from late spring through early summer (Hoffman, 1969). They are composed of highly folded anastomosing cords of epithelial cells. During the active male phase (winter, spring and early summer), cytoplasmic atrophy becomes obvious and vacuolated cells make their appearance in androgenic glands. Cytoplasmic atrophy is characterised by the presence of elongated cells with flattened nucleus, increased cytoplasmic volume, and a greater affinity for basic stains. The vacuolated cells increase during early summer months; they have a swollen nucleus and show less affinity to basic stains. This stage is immediately followed by a stage of cellular breakdown in which totally degenerated cytoplasm and unstainable nuclei are seen. During late summer and early autumn months, when transitional males are present, the cells of androgenic glands show pycnotic nuclei and large cytoplasmic inclusions. Towards the last phase of autumn and in early winter, the androgenic glands

of P. platyceros are comprised of only empty cords. Barring Gomori's acid phosphatase-positive granules (lysosomes?), no other cytoplasmic inclusions have been seen in androgenic glands of P. platyceros. These granules are present in large numbers in the stage of cytoplasmic atrophy during the male phase; it may well be that the presence of these granules in such numbers heralds the beginning of cell death (Hoffman, 1969).

A round-the-year analysis of the protein content of the vas deferens and of the 'vas deferens factor' (Vdf) of O. platytarsis reveals that the fluctuations in both the variables are comparable (Fig. 5.9). Both values are at their maximum during August. Vdf is at its lowest during November, whereas protein content dropped to its lowest value during December. Both Vdf and protein content in vas deferens show another peak during April/May. In the same way, we have found that variations in 'CSG-factor' (Glf) and in percentage of protein in CSG can be correlated (Fig. 5.10): Glf and the percentage of protein in CSG reach their peak values during August and their lowest values during October. It may be recalled here that in Paratelphusa hydrodromous protein (which is chief biochemical component of semen) and Vdf attain their highest values during August/September, when adult males are sexually the most active during the second half of the year

(Mathad, 1983). The protein content of semen of the barnacle Balanus balanus also shows variations as is evident from an eleven-week sampling study by Barnes and Blackstock (1974).

A comparison of the protein content of the CSG of O. platytarsis during five different periods (March/April, i.e. summer; June/July, i.e. monsoon; August, i.e. postmonsoon; October/November, i.e. early winter; and December/January, i.e. late winter) clearly reveals that the percentage of protein in CSG during August is significantly higher than during all other sampling periods (Table 5.2) and is at its lowest during early winter. Fluctuation in protein content of the vas deferens through various seasons in comparison is much less prominent in O. platytarsis (Table 5.7). In CSG during summer, the FAA content is significantly higher than during other sampling periods; during late winter FAA levels dropped to their minimum (Table 5.3). Variations in FAA content of the vas deferens did not follow any given pattern; instead, it changed abruptly during different sampling periods (Table 5.8). Marked fluctuations in FAA content of the vas deferens (and semen) have been also found in P. hydrodromous (Mathad, 1983), but in that species such variations are rather uniform and the highest values are obtained during August/September. In Balanus balanus

(Barnes and Blackstock, 1974), seasonal variations in individual amino acids are said to be insignificant, but the authors have not clarified it statistically. In Balanus semen, there is no specific trend in variation of individual amino acids, the variation being common to all (Barnes and Blackstock, 1974).

There is a marked, but gradual, fluctuation in oligosaccharide fraction of the vas deferens of O. platytarsis. The percentage of oligosaccharide fraction is significantly higher during August than other seasons. There is also a significant decline in oligosaccharide content of the vas deferens during early winter (Table 5.9). As in the case of proteins, the oligosaccharide content of the CSG registers peak values in August (Table 5.4). The percentage of oligosaccharide fraction in CSG falls from September through April and is at its lowest during June/July. On the contrary, the percentage of polysaccharides in CSG is nearly constant throughout the sampling periods (Table 5.5), whereas in the vas deferens, except for a peak registered during late winter (which is significantly higher than values of other seasons), variations in polysaccharides have been found to be not significant (Table 5.10). As a rule, lipid contents of the CSG and of the vas deferens remained nearly steady during the various sampling periods (Tables 5.6 and 5.11).

Biochemical data on vas deferens and CSG of O. platytarsis, presented in this chapter, reveal that the seasonal variations in different components do not follow any definite trend and are difficult to interpret. Nevertheless, protein and oligosaccharide contents of CSG and vas deferens reach peak values during August, when Glf and Vdf are also at their maximum suggesting that this may be a peak period of male reproductive activity in O. platytarsis. But the polysaccharide and lipid contents show little variation during different seasons. Fluctuation in FAA, which forms only a small percentage of the total organic constituents of the male reproductive system, follows different patterns in CSG and vas deferens, the significance of which is not understood.

Our studies have shown that the changes in male reproductive system of O. platytarsis can be correlated with the activity of the androgenic gland. The androgenic gland of O. platytarsis does not degenerate completely, nor are spermatozoa totally absent at any time in the testis: the vas deferens and CSG tubules of O. platytarsis are always filled with seminal secretion. Spermatidogenesis and spermiogenesis as well as the synthetic activity of epithelial cells of the vas deferens and CSG tubules take place largely during the first half of the year, when the

androgenic gland cells are 'healthy' and the titre of the androgenic hormone in circulation is apparently low. Towards the middle of the year or early part of the second half of the year, androgenic gland cells undergo degenerative changes, and from assumptions based on histology, the levels of circulating androgenic hormone are high during this period. Vdf, Glf and the chief biochemical components of the vas deferens and CSG increase during this period, reaching a peak in August. By early winter, the androgenic gland enters the inactive phase, and Glf and Vdf begin to decrease. In male Paratelpusa hydrodromous, the biochemical components of the testis, vas deferens and semen vary in accordance with the mating season of the animal. It remains to be shown whether there exists a comparable relationship between biochemistry of the male reproductive system and mating in also O. platytarsis. Barnes and Blackstock (1974) reported the presence of a tripeptide in Balanus semen that sharply increases in concentration before copulation.

5.5. SUMMARY

1. Studies on mature O. platytarsis (in intermoult) have shown that the activity of the male reproductive system fluctuates within small limits during different seasons. The vas deferens and CSG tubules of this species have been found filled with secretions

throughout the sampling periods.

2. The relative abundance of different stages in spermatogenesis shows fluctuations in relation to different seasons.
3. In O. platytarsis, individual spermatogonia are not encircled by mesoderm cells. Secondary spermatocytes exist in 2 stages, the resting phase (ScR) and the other undergoing division (ScD). Spermatids have been observed in early (SpE) and late (SpL) stages. The quantity of PAS-positive substances is relatively more in spermatids than in spermatogonia or spermatocytes of O. platytarsis.
4. Percentual distribution in testis of SpE and SpL shows much variations during different seasons. Though the process of spermatogenesis occurs during all seasons, the progress of spermatogenesis and production of spermatozoa are not uniform throughout the sampling periods. Output of spermatozoa and other spermatogenic processes appear to be at their highest during the first half of the year.
5. During January-April, the volume of androgenic gland (AG) of O. platytarsis was found to be more. During January/February the cytoplasmic content of AG cells was high, nuclei were large with prominent

nucleoli; and degenerated cells were scarce. During June/July the volume of AG was less, cytoplasm more compact, and pycnotic nuclei were more common.

During September/October AG was found to shrink further in size and to consist of only a few cords of small cells, with little cytoplasm and indistinct nuclei. In O. platytarsis, the mode of secretion of AG appears to be holocrine.

6. Fluctuations in protein content of the vas deferens and in 'vas deferens factor' (Vdf) are comparable, both values being at their maximum during August. Similarly, variations in 'CSG-factor' (Glf) and percentage of protein in CSG could be correlated.
7. Biochemical data on vas deferens and CSG of O. platytarsis reveal that the seasonal variations in different components do not follow any definite trend.
8. The percentage of protein in CSG during August is significantly higher than that during other seasons.
9. There was a gradual fluctuation in oligosaccharide fraction of the vas deferens of O. platytarsis. The oligosaccharide content of both vas deferens and CSG reached peak levels in August. Though

polysaccharide content of the vas deferens registered peak value in late winter, its fluctuation during other seasons was negligible as in CSG. Lipids in CSG and vas deferens remained almost steady during the various seasons.

10. Fluctuations in FAA follow different patterns in CSG and vas deferens. In CSG, for instance, during summer, the FAA content was significantly higher than during other sampling periods. In vas deferens, the percentage of FAA changed abruptly during different sampling periods.

Chapter VI

Endocrine control of reproduction in male *Ocypode Platytarsis* Milne Edwards

M Sukumaran “Certain aspects of reproductive biology of the ghost crab, *Ocypode Platyt Aris* with special reference to the male” Thesis. Department of Zoology, University of Calicut, 1985

6.1. INTRODUCTION

Control mechanisms involved in production of secretions by the male duct in crustaceans are only poorly understood. Recent investigations suggest that a relationship exists between activity of the endocrine glands and male reproductive physiology in crustaceans (K.G. Adiyodi and R.G. Adiyodi, 1970; K.G. Adiyodi, 1982; R.G. Adiyodi, 1985; Charniaux-Cotton et al., 1985). Eyestalk principles, long considered to be inhibitory to reproductive and somatic growth (K.G. Adiyodi and R.G. Adiyodi, 1970), are now being recognized to be not totally inhibitory, but to have only a restraining influence on these processes (Anilkumar and K.G. Adiyodi, 1980; Mathad and K.G. Adiyodi, 1981, Mathad, 1983; R.G. Adiyodi, 1985); the principles probably in decreasing titres, are essential to ensure normal reproductive and somatic growth. Though it is well established that male reproductive activity in crustaceans is controlled by a male sex hormone (androgenic hormone) (Charniaux-Cotton et al., 1985), the exact role of eyestalk principles on activity of the androgenic glands which produce this hormone, or on male reproductive physiology is not clear. It may well be that effects of eyestalk principles on male reproductive activity in crustaceans are not mediated through the androgenic

gland. In juvenile Penaeus japonicus, eyestalk ablation leads to precocious development of the androgenic glands in a week (Payen et al., 1981), whereas in adult Rhithropanopeus harrisi, there is no significant modification in androgenic gland cells following the same operation (Payen et al., 1971). In the protandric shrimp Pandalus platyceros, the eyestalk inhibition of androgenic glands is seasonal (Hoffman, 1968). These observations suggest that eyestalk influences on androgenic gland activity may be modulated by species, age and season.

It is clear that the male reproductive system and semen of O. platytarsis is peculiar in many respects (e.g., composition of seminal plasma, presence of collateral glands, etc.) (Chapter III); moreover, the androgenic gland activity as well as the quantity of biochemical components (proteins, FAA and carbohydrates) of the vas deferens and CSG show fluctuations during different seasons (Chapter V). We thought that it would be worthwhile, therefore, to investigate the control mechanisms involved in semen production and androgenic gland activity of this species. In this chapter, we summarize the results of destalkation and eyestalk implantation experiments on O. platytarsis with a view to find out the effects of eyestalk hormones and androgenic

hormones on the activity of ductual and collateral glands of the sperm duct of male O. platytarsis.

6.2. MATERIALS AND METHODS

6.2.1. Eyestalk Ablation Experiments

O. platytarsis, used in this investigation (cw 5.0 to 5.3 cm), were collected from Calicut beach. Prior to the experiment, they were reared in the laboratory for a week and fed ad lib. during this period on fresh ox liver. Only the healthy ones which had successfully acclimatized themselves to conditions in our culture room were selected for experiment. The crabs were divided into three groups. From the first two groups both the eyestalks were simultaneously removed using a pair of sterilized scissors. After sealing the wound with sterilized cotton and plaster, a coating of modelling clay was applied to minimize bleeding. Care was taken to rear such animals individually as they became increasingly cannibalistic. The first group of destalked crabs (group-I) served as the experimentals. The second group of destalked crabs (group-II) were given injections of saline extracts of two eyestalks, twice a week. The eyestalk extract was prepared from the required number of stalked eyes, obtained from male crabs. For this purpose, the chitinous exoskeleton and ommatidia on the

eyes were first removed from the stalked eyes; the soft tissue containing the neurosecretory cells and sinus glands was isolated, ground with 2 ml of 0.65% saline and centrifuged for 10 min. at 1,500 rpm; the supernatant alone was used for injection. Mortality rate of destalked crabs injected with eyestalk extract (group-II crabs) was high; they were, therefore, reared only for 20 days postoperation. The third group of crabs (group-III) were composed of normal males with intact eyestalks.

The required number of group-I, group-II and group-III crabs were sacrificed 10 and 20 days postoperation and the histological changes in male reproductive system and androgenic glands were analysed. The total wet weight and weight of biochemical components (proteins, oligosaccharide fraction, polysaccharides and free amino acids) of vas deferens (AVD+MVD) and coral-shaped accessory sex gland (CSG) of the experimentals were also compared statistically with those of the controls. The remaining group-I crabs were reared until moulting, when they were sacrificed along with the required number of group III animals.

The male genital duct and CSG were carefully dissected out from both experimental (group-I) and control (group-II and group-III) crabs and the total wet weight of each tissue was directly read using a single pan

automatic electronic balance. Samples of various parts of the male reproductive system and androgenic glands were fixed in 10% saline-formol or Bouin's fixing fluid. Paraffin sections (5-6 μ m thick) of both tissues were cut and stained in Heidenhain's haematoxylin and alcoholic eosin. Some sections of the androgenic glands were stained in chrome-haematoxylin-phloxin.

The total TCA-precipitable proteins, free amino acids (FAA), oligosaccharide fraction and polysaccharides of the vas deferens and CSG of experimental as well as control animals were estimated using standard biochemical techniques, described earlier (Section 4.2.2). Moulting stages of the crabs were determined using the fourth maxillipede as with pleopods of females (after Drach, 1939; see also Anilkumar, 1980).

6.2.2. Implantation Experiments

CSG was dissected out from normal crabs with their eyestalks intact and cultured in vivo in haemocoel of male and female O. platytarsis. For implantation of the gland, a small window (5x5 mm) was made on the carapace (1/3rd removed from the antero-lateral end) using a sharp blade and the CSG was next carefully inserted into the haemocoel through the opening. The orifice was neatly closed by placing the carapace lid in place

and covering it with a piece of plaster. After 10 days, the implants were recovered and fixed in 10% saline-formol for histological observations.

6.3 RESULTS

We have already seen that there are distinct fluctuations in activity of the male reproductive system of O. platytarsis during different seasons (Section 5.3). In order to clarify the role, if any, of eyestalk principles on male reproductive physiology of O. platytarsis, two sets of eyestalk ablation experiments were designed by us, one during September/October (when androgenic glands were small, contained vacuolated cells and were apparently inactive), and the other during March/April (when androgenic glands were large, contained 'healthy' cells and were apparently active).

Eyestalk ablation in O. platytarsis led to an astonishing increase in appetite. Unless fed liberally, the rate of mortality in destalked crabs was rather high. Destalked individuals ($cw = 5.0-5.3$ cm) consumed as much as 3-4 gm of fresh^{ox} liver a day, whereas intact animals of the same size did not consume more than 2.0-2.5 gm a day. The intensity of hyperphagia, however, declined gradually beginning a few days after eyestalk ablation; the crabs finally stopped to feed, and within a few

days the destalked crabs had moulted.

6.3.1. Effect of Eyestalk Ablation on CSG of *O. platytarsis* during September/October

By 10 days postoperation, the CSG tubules of destalked animals did not differ significantly in cytology from those of the controls (Fig.6.5). However, by 20 days postoperation, the destalked crabs had already reached stage D_0 - D_1 and cytology of the secretory epithelium lining the CSG tubules betrayed an appreciable degree of activity. This was evident from an increase in the height of the epithelium (Table 6.33) as well as from a significant enlargement of the epithelial cell nuclei (Fig. 6.6). Though no cytologically detectable rise in quantity of glandular secretion was in evidence, 20 days postoperation, an analysis of the total TCA-precipitable protein content of the CSG in destalked crabs revealed a statistically significant rise in protein in comparison to the controls (Table 6.14). However, in crabs which received injections of eyestalk extracts, there was no such increase in protein content of the CSG (Tables 6.27 and 6.28), nor could we observe increased secretory activity of the glandular epithelium. The rise in protein content of CSG of eyestalkless crabs was evident at the time of moulting also (Table 6.15).

The FAA content of the CSG of destalked crabs was significantly higher than that of controls even by 10 days postoperation (Table 6.13). A further increase in FAA level was observed in the CSG as the eyestalkless crabs entered stage E of the moulting cycle (Tables 6.15, 6.17 and 6.18). The oligosaccharide fraction and polysaccharide of CSG, however, did not register a corresponding increase in eyestalkless crabs during September/October (Tables 6.13 to 6.15).

6.3.2. Effect of Eyestalk Ablation on the Vas Deferens of *O. platytarsis* during September/October

Bilateral eyestalk ablation during September/October produced little perceptible effect on the vas deferens by 10 days postoperation. Histological picture of the vas deferens of 10 day-destalked crabs was comparable to that of the controls (Fig. 6.1). The epithelium of AVD-I measured about $2.31\ \mu\text{m}$ and of AVD-II about $17.7\ \mu\text{m}$ in height, whereas AVD-III epithelium was only about $6.5\ \mu\text{m}$. The epithelial layers of MVD-I ($5.2 \pm 4.06\ \mu\text{m}$), MVD-II ($50.4 \pm 4.3\ \mu\text{m}$) and the ejaculatory duct (ED) ($12.6 \pm 2.1\ \mu\text{m}$) of 10 day-destalked crabs were comparable to those of normal crabs.

However, by 20 days postoperation, changes in cytological features were apparent almost throughout

the male genital duct. For instance, the nature of the secretory epithelium of AVD-I showed that cytological changes have taken place as evidenced by the increase in height of the epithelium and by the signs of increased nuclear activity. There were, however, no secretory granules in cytoplasm of the epithelial cells of AVD-I, though substantial quantities of SE-1 could be detected in the lumen. Twenty days postoperation, the secretory epithelium of AVD-II also responded by an appreciable increase in cell size, but the nuclei surprisingly were in a highly pycnotic condition. The cytoplasm was not only bereft of any detectable secretory granules but also showed signs of vacuolization. Surface of the epithelial cells became highly uneven, giving a characteristic folded appearance (Fig. 6.2). Hypertrophy of the secretory epithelial cells was evident in AVD-III also, but nuclear pycnosis and cytoplasmic vacuolization were observed only in AVD-II. Epithelial cells of MVD-I and MVD-II exhibited little signs of change in cytology in 20 day-destalked animals, but ED epithelial cells did show some increase in height (Table 6.33). In destalked crabs which had subsequently received injections of eyestalk extracts, the histology of the vas deferens differed little from that of normal animals with their eyestalks intact. -

The cytological picture of the epithelial cells of vas deferens in destalked crabs at the time of moulting (27-35 days postoperation) was almost comparable to that of the control animals (group-III). The cells were healthy and no nuclear pycnosis was observed.

6.3.3. Effect of Eyestalk Ablation on the CSG of O. platytarsis during March/April

The epithelial cells of CSG tubules of destalked crabs, 10 and 20 days postoperation during March/April, were comparable to that of the destalked crabs during September/October. However, the secretory epithelial cells of CSG became vacuolated as the destalked crabs entered stage -E (Fig. 6.7) (23-25 days postoperation). In male O. platytarsis destalked during March/April, there was no increase in wet weight of the CSG, but the FAA and oligosaccharide fraction did register a significant increase, by 10 days postoperation (Table 6.7), compared to controls (group-III). By 20 days postoperation, when the crabs reached stage D₂-D₃ of the moulting cycle, the TCA-precipitable proteins ($t=3.93$; $p < 0.005$), and wet weight ($t=6.83$; $p < 0.001$) of CSG increased significantly; carbohydrate content (oligosaccharide fraction + polysaccharides) of the CSG of eyestalkless crabs was also significantly more than that of the controls. Interestingly, when eyestalkless crabs reached stage-E (23-25 days

postoperation), there was a significant decrease in wet weight of the CSG, followed by a fall in total proteins (Table 6.12).

6.3.4. Effect of Eyestalk Ablation on Vas Deferens of O. platytarsis during March/April

During March/April, the vas deferens of destalked crabs was histologically not very different from that of the controls by 10 days postoperation. Epithelial cells of the anterior vas deferens (AVD-I, AVD-II and AVD-III) of both the controls and the experimentals were filled with cytoplasm, and had large nuclei (Fig.6.3); the cytoplasmic basophilia was intense in both. By 20 days, epithelial cells of the AVD of destalked crabs had gained as much height as destalked crabs during September/October; however, the peculiar irregular margin of epithelial cells of AVD-II characteristic of destalked O. platytarsis during September/October, did not appear during March/April on destalking (Fig.6.4). The lumen of AVD-II was rich in SE-1. In some instances, vacuoles were present in cytoplasm of the epithelial cells of AVD-II. The epithelia of MVD-II and ED of destalked crabs were comparable in histology to that of the controls. As the destalked crabs moulted (23-25 days postoperation) during March/April, the epithelial cells of AVD-II presented histological pictures reminiscent of those of 20-day postoperated

crabs during September/October; what was noteworthy, however, was that pycnotic nuclei were comparatively few.

Tables 6.1 to 6.6 show the changes in vas deferens of crabs at various intervals after bilateral eyestalk ablation. The results reveal that wet weight of the vas deferens increased significantly by 10 days postoperation, and that this increase was maintained until moulting. The protein content of the vas deferens of eyestalkless crabs also showed a similar tendency. However, the FAA content did not show any such increase, even 22-25 days postoperation, when the eyestalkless crabs reached stage E.

By 20 days postoperation the oligosaccharide fraction of the vas deferens of destalked crabs was significantly higher than that of the controls; it was, however, comparable to that of the 10-day-postoperated crabs (Table 6.4). When the destalked crabs reached stage E (23-25 days postoperation), the oligosaccharide fraction of the vas deferens was significantly higher than that of the controls ($t=3.11$; $p < 0.02$) (Table 6.3) and that of 10-day destalked crabs ($t=2.92$; $p < 0.02$) (Table 6.5). Polysaccharides did not show any statistically significant variation even 23-25 days postoperation.

Data on wet weight and various biochemical components of the vas deferens and CSG of destalked crabs that had reached premoult stage of the moulting cycle and normal crabs (eyestalks intact) that were in premoult are given in Tables 6.29 to 6.32 for comparison.

6.3.5. Effect of Eyestalk Ablation on Androgenic Glands of *O. platytarsis*

As stated earlier (Section 5.3.2) the androgenic glands of *O. platytarsis* are large during March/April, with healthy cells filled with cytoplasm. The nuclei are large and show distinct chromatin blocking (Fig. 6.10). Bilateral eyestalk ablation during this season leads to remarkable changes in histology of the androgenic glands. The gland cells become smaller and vacuolated; degenerated areas and pycnotic nuclei are common (Fig. 6.11). By 23-25 days postoperation, when crabs moult, the androgenic glands are still small and the cells become highly vacuolated with pycnotic nuclei (Fig. 6.12).

During September/October, eyestalk ablation did not produce any cytologically detectable changes in androgenic glands of *O. platytarsis* even 27 to 35 days postoperation, when the destalked crabs moulted. The degree of general nuclear pycnosis and cytoplasmic vacuolization of experimental animals was comparable to that of controls.

6.3.6. Role of Androgenic Gland in Maintenance of CSG of
O. platytarsis

CSG was implanted into the haemocoel of male (having androgenic hormone in the blood) and female (deprived of androgenic hormone) O. platytarsis. The implants were removed after 10 days and examined histologically. They were surrounded by cells from neighbouring tissues, in both male and female crabs. In implants retrieved from female crabs (sectioned and stained with Heidenhain's haematoxylin and alcoholic eosin), it was not possible to identify the CSG tubules; the original shape of the secretory tubules as well as the fibrous nature of the secretion was not distinct (Fig. 6.9). In implants retrieved from male crabs, the secretory epithelium of CSG tubules was not histologically distinct, but the fibrous secretion in CSG tubules was found to keep its identity over the period of the experiment (Fig. 6.8).

Attempts to remove the androgenic glands from the male O. platytarsis were not successful.

Figs. 6.1 - 6.4. Photographs of cross sections of AVD to show the nature of its epithelial lining in experimental (group-I) and normal (control) O. platytarsis.

Fig. 6.1 Control (AVD-II) (September)

Formol-saline, Heidenhain's haematoxylin-alcoholic eosin X 380

Fig. 6.2 Experimental (AVD-II, 20 days postoperation, October).

Formol-saline, Heidenhain's haematoxylin-alcoholic eosin X 450

Fig. 6.3 Experimental (AVD-I, 20 days postoperation, April).

Formol-saline, Heidenhain's haematoxylin-alcoholic eosin X 450

Fig. 6.4 Experimental (AVD-II, 20 days postoperation April).

Formol-saline, Heidenhain's haematoxylin-alcoholic eosin X 270

E - epithelium; N - nuclei; Np - pycnotic nuclei, SE-1 - see Section 3.3.3



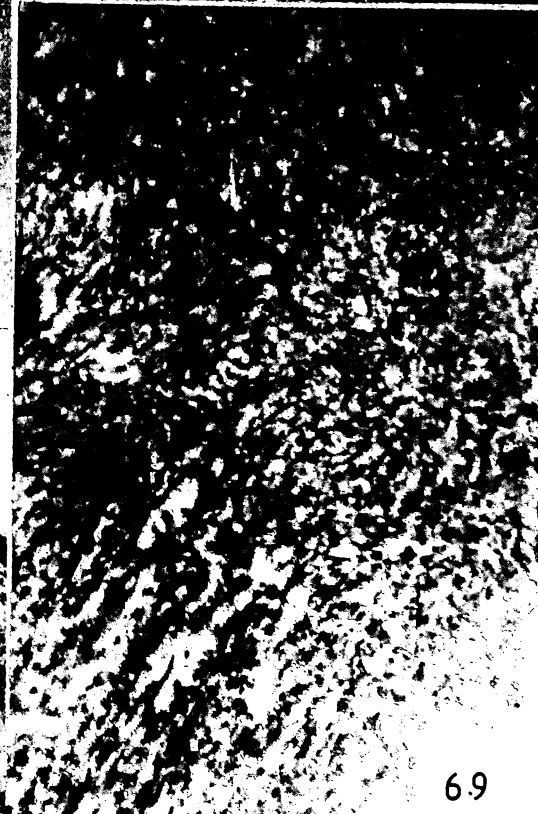
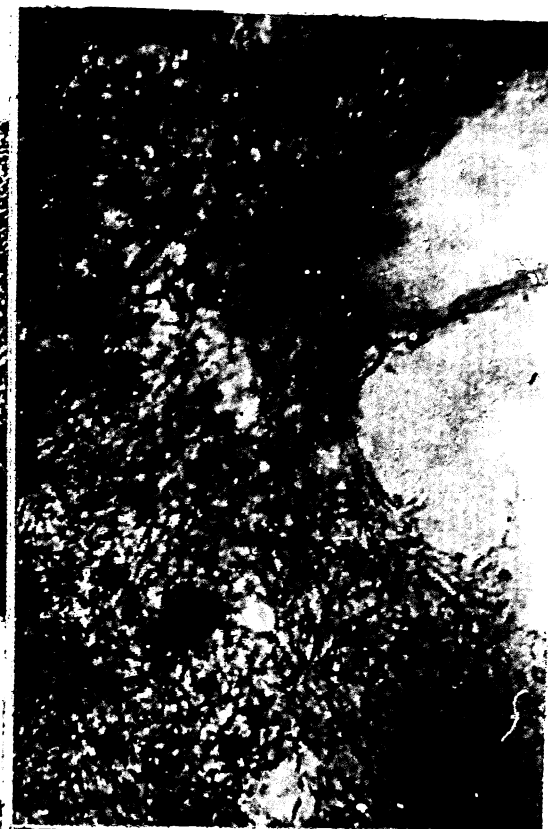
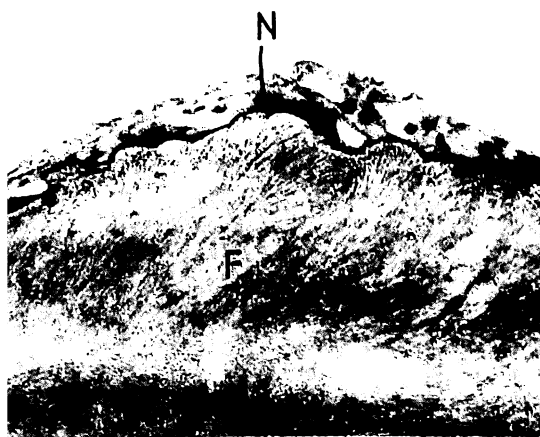
Figs. 6.5 - 6.7. Photographs showing the epithelial lining of CSG of experimental (group-I) and normal (control) O. platytarsis

Fig. 6.5 Control (September)
Formol-saline, Heidenhain's haematoxylin-
alcoholic eosin X 230

Fig. 6.6 Experimental (20 days postoperation, October)
Formol-saline, Heidenhain's haematoxylin-
alcoholic eosin X 220

Fig. 6.7 Experimental (20 days postoperation, April)
Formol-saline, Heidenhain's haematoxylin-
alcoholic eosin X 210

Fig. 6.8 - 6.9. Sections through implants of CSG of O. platytarsis retrieved respectively from male and female recipients.
Note the fibrous nature (arrow) of CSG secretion in fig. 6.8).
Formol-saline, Heidenhain's haematoxylin-
alcoholic eosin X 500.
E - epithelium; F - fibrous secretion;
N - nucleus



Figs. 6.10 - 6.12. Photographs showing sections of androgenic gland of experimental (group-I) and normal (control) O. platytarsis during March/April.

Fig. 6.10 Control

Formol-saline, Heidenhain's haematoxylin-alcoholic eosin X 300

Fig. 6.11 Experimental (20 days postoperation)

Note vacuolization of cells and the appearance of degenerated areas and pycnotic nuclei.

Formol-saline, Heidenhain's haematoxylin-alcoholic eosin X 400

Fig. 6.12 Experimental (30 days postoperation)

Formol-saline, Heidenhain's haematoxylin-alcoholic eosin X 380.

N - nucleus; Np - pycnotic nuclei.

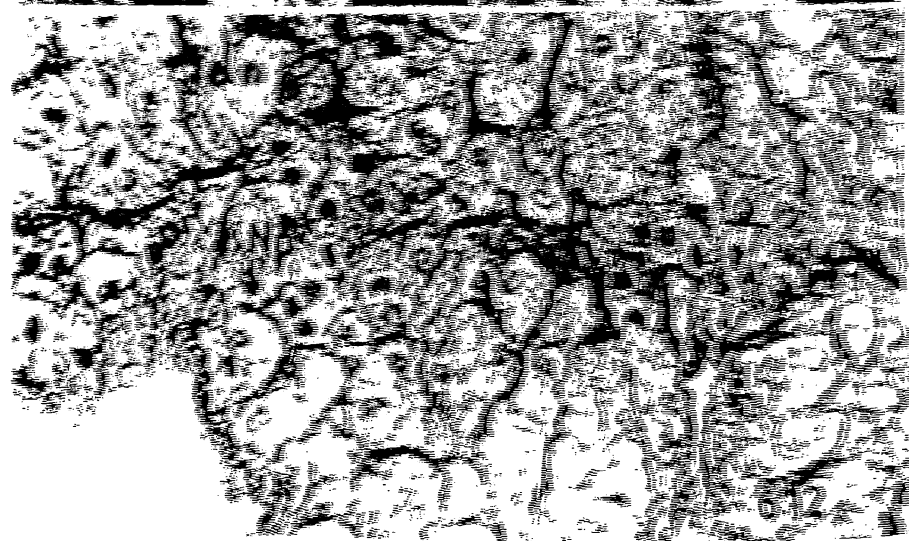
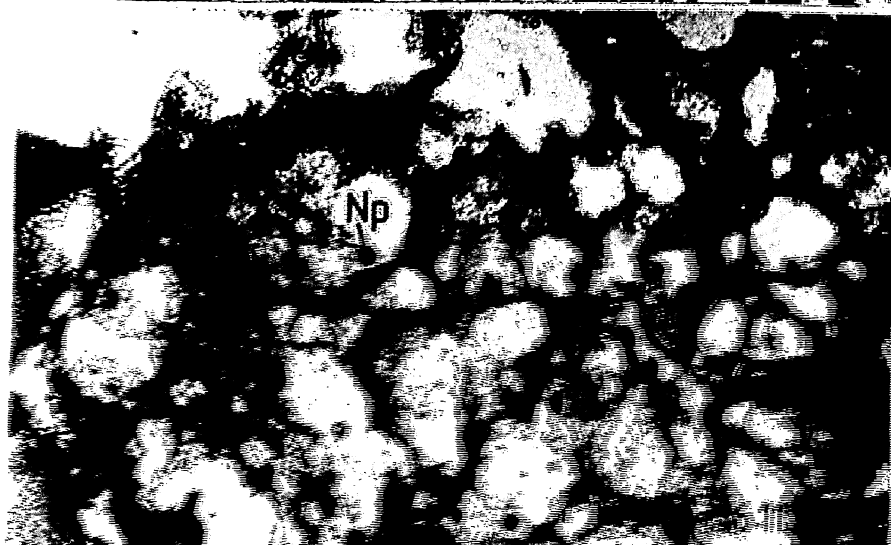
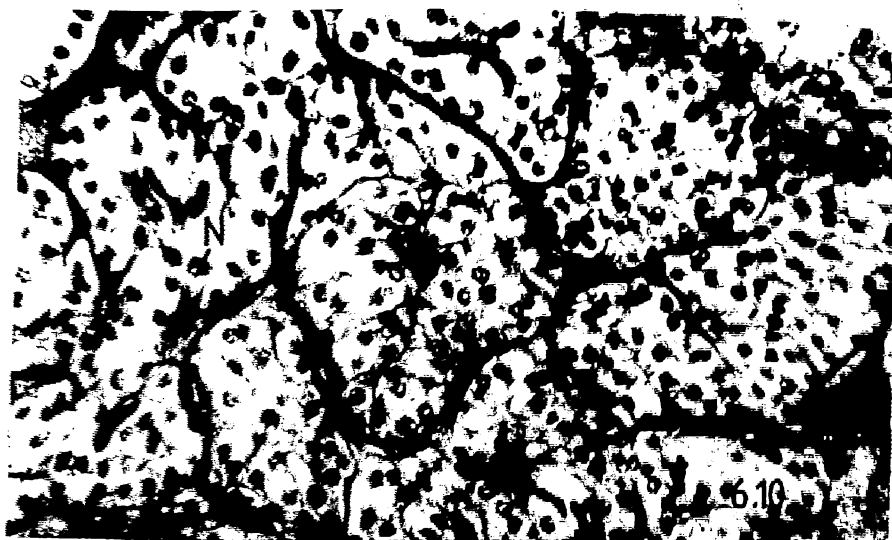


Table 6.1

Effect of eyestalk ablation on O. platytarsis: changes in composition of the vas deferens during March/April, 10 days postoperation (quantity expressed as mg/individual, $\bar{X} \pm \text{SD}$).

	Destalked (n=5)	Controls* (n=5)	t	P
Moulting stage				
TCA-precipitable proteins	C ₃ -C ₄ 29.39 $\pm$ 2.10	C ₃ -C ₄ 21.84 $\pm$ 4.19	3.60	<0.01
Free amino acids (FAA)	0.49 $\pm$ 0.06	0.456 $\pm$ 0.14	0.499	NS
Oligosaccharide fraction	0.677 $\pm$ 0.074	0.51 $\pm$ 0.069	3.69	<0.01
Polysaccharides	0.465 $\pm$ 0.05	0.547 $\pm$ 0.21	0.878	NS
Wet weight	262 $\pm$ 40.1	181 $\pm$ 43.64	3.06	<0.02

* group-III

Table 6.2

Effect of eyestalk ablation on O. platytarsis: changes in composition of the vas deferens during March/April, 20 days postoperation (quantity expressed as mg/individual, $\bar{X} \pm \text{SD}$).

	Destalked (n=5)	Controls* (n=5)	t	p
Moulting stage	D ₂ -D ₃	C ₃ -C ₄		
TCA-precipitable proteins	31.46 $\pm$ 6.53	23.14 $\pm$ 2.29	2.69	< 0.05
Free amino acids (FAA)	0.85 $\pm$ 0.44	0.683 $\pm$ 0.042	0.845	NS
Oligosaccharide fraction	0.708 $\pm$ 0.092	0.507 $\pm$ 0.032	4.62	< 0.001
Polysaccharides	0.841 $\pm$ 0.39	0.503 $\pm$ 0.19	1.74	NS
Wet weight	276 $\pm$ 84	180.33 $\pm$ 27.13	2.43	< 0.05

* group-III

NS - not significant

Table 6.3

Effect of eyestalk ablation on O. platytarsis: changes in composition of the vas deferens during March/April, at moulting after destalking (quantity expressed as mg/individual, $\bar{X} \pm SD$).

	Destalked (n=5)	Controls* (n=5)	t	p
Moulting stage	E	C3-C4		
TGA-precipitable proteins	34.52 $\pm$ 6.55	23.41 $\pm$ 4.6	3.11	< 0.02
Free amino acids (FAA)	0.49 $\pm$ 0.20	0.688 $\pm$ 0.06	1.91	NS
Oligosaccharide fraction	0.73 $\pm$ 0.091	0.59 $\pm$ 0.043	3.11	< 0.02
Polysaccharides	0.557 $\pm$ 0.09	0.515 $\pm$ 0.31	0.291	NS
Wet weight	308 $\pm$ 102	200.67 $\pm$ 16.17	2.33	< 0.05

* group-III

NS - not significant

Table 6.4

Effect of eystalk ablation on O. platytarsis: changes in composition of the vas deferens of crabs destalked during March/April, at 10 and 20 days postoperation (quantity expressed as mg/individual, $\bar{X} \pm SD$).

	Destalked 10 days postoperation (n=5)	Destalked 20 days postoperation (n=5)	t	p
Moulting stage				
TCA-precipitable proteins	C3-C4	D2-D3		
Free amino acids (FAA)	29.39 $\pm$ 2.1	31.46 $\pm$ 6.53	0.675	NS
Oligosaccharide fraction	0.49 $\pm$ 0.06	0.85 $\pm$ 0.44	1.81	NS
Polysaccharides	0.677 $\pm$ 0.074	0.708 $\pm$ 0.092	0.588	NS
Wet weight	0.465 $\pm$ 0.05	0.841 $\pm$ 0.39	2.14	NS
	262 $\pm$ 40.1	276 $\pm$ 84	0.337	NS
NS - not significant				

Table 6.5

Effect of eyestalk ablation on O. platytarsis: changes in composition of the vas deferens of crabs destalked during March/April, at 10 days postoperation and at moulting after destalking (quantity expressed as mg/individual, $\bar{X} \pm \text{SD}$).

	Destalked 10days postoperation (n=5)	Destalked at moulting (n=5)	t	p
Moulting stage				
TCA-precipitable proteins	C ₃ -C ₄ 29.39 $\pm$ 2.1	E 34.52 $\pm$ 6.55	1.67	NS
Free amino acids (FAA)	0.49 $\pm$ 0.06	0.488 $\pm$ 0.20	0.02	NS
Oligosaccharide fraction	0.577 $\pm$ 0.074	0.73 $\pm$ 0.091	2.92	< 0.02
Polysaccharides	0.465 $\pm$ 0.05	0.557 $\pm$ 0.09	1.99	NS
Wet weight	265 $\pm$ 40.1	308 $\pm$ 102	0.939	NS
NS - not significant				

Table 6.6

Effect of eyestalk ablation on O. platytarsis: changes in composition of the vas deferens of crabs destalked during March/April, at 20 days postoperation and at moulting after destalking (quantity expressed as mg/individual, $\bar{X} \pm \text{SD}$).

	Destalked 20 days postoperation (n=5)	Destalked at moulting (n=5)	t	p
Moulting stage	D ₀ -D ₂	E		
TCA-precipitable proteins	31.46 $\pm$ 6.53	34.52 $\pm$ 6.55	0.74	NS
Free amino acids (FAA)	0.85 $\pm$ 0.44	0.488 $\pm$ 0.2	1.68	NS
Oligosaccharide fraction	0.708 $\pm$ 0.092	0.73 $\pm$ 0.091	0.380	NS
Polysaccharides	0.841 $\pm$ 0.39	0.557 $\pm$ 0.09	1.59	NS
Wet weight	276 $\pm$ 84	308 $\pm$ 102	0.542	NS
NS - not significant				

Table 6.7

Effect of eyestalk ablation on O. platytarsis: changes in composition of the CSG during March/April, 10 days postoperation (quantity expressed as mg/individual, $\bar{X} \pm$ SD).

	Destalked (n=5)	Controls* (n=5)	t	p
Moulting stage	C ₃ -C ₄	C ₃ -C ₄		
TCA-precipitable proteins	29.05 $\pm$ 6.1	23.61 $\pm$ 2.6	1.43	NS
Free amino acids (FAA)	1.44 $\pm$ 0.29	0.649 $\pm$ 0.12	4.87	< 0.002
Oligosaccharide fraction	1.76 $\pm$ 0.34	0.892 $\pm$ 0.31	3.60	< 0.01
Polysaccharides	0.37 $\pm$ 0.20	0.26 $\pm$ 0.09	0.879	NS
Wet weight	314 $\pm$ 72.2	212 $\pm$ 32	2.26	NS

* group-III

NS-not significant

Table 6.8

Effect of eyestalk ablation on O. platytarsis: changes in composition of the CSG during March/April, 20 days postoperation (quantity expressed as mg/individual $\bar{X} \pm$ SD).

	Destalked (n=5)	Controls* (n=5)	t	p
Moulting stage	D ₂ -D ₃	C ₃ -C ₄		
TCA-precipitable proteins	33.33 $\pm$ 5.06	22.78 $\pm$ 3.20	3.93	< 0.005
Free amino acids (FAA)	0.885 $\pm$ 0.25	0.658 $\pm$ 0.19	1.62	NS
Oligosaccharide fraction	1.79 $\pm$ 0.44	0.933 $\pm$ 0.30	3.60	< 0.01
Polysaccharides	0.616 $\pm$ 0.05	0.311 $\pm$ 0.08	7.23	< 0.001
Wet weight	400 $\pm$ 38	197 $\pm$ 51.08	6.87	< 0.001

* group-III

NS - not significant

Table 6.9

Effect of eyestalk ablation of O. platytarsis: changes in composition of CSG during March/April, on moulting after destalking (quantity expressed as mg/individual, $\bar{X} \pm SD$).

	Destalked (n=5)	Controls* (n=5)	t	p
Moulting stage	E	C ₃ -C ₄		
TCA-precipitable proteins	22.25 $\pm$ 2.26	19.996 $\pm$ 5.01	0.826	NS
Free amino acids (FAA)	1.0 $\pm$ 0.012	0.925 $\pm$ 0.021	0.710	NS
Oligosaccharide fraction	1.43 $\pm$ 0.45	1.152 $\pm$ 0.09	1.23	NS
Polysaccharides	0.477 $\pm$ 0.19	0.234 $\pm$ 0.22	1.67	NS
Wet weight	253 $\pm$ 59	203.5 $\pm$ 31.81	1.65	NS
* group-III				
NS - not significant				

Table 6.10

Effect of eyestalk ablation on O. platytarsis: changes in composition of CSG of crabs destalked during March/April, at 10 days and 20 days postoperation (quantity expressed as mg/individual, $\bar{X} \pm \text{SD}$).

	Destalked 10 days postoperation (n=5)	Destalked 20 days postoperation (n=5)	t	p
Moulting stage	C ₃ -C ₄	D ₂ -D ₃		
TGA-precipitable proteins	29.05 $\pm$ 6.1	33.33 $\pm$ 5.06	1.21	NS
Free amino acids (FAA)	1.44 $\pm$ 0.29	0.885 $\pm$ 0.25	3.24	< 0.02
Oligosaccharide fraction	1.76 $\pm$ 0.34	1.79 $\pm$ 0.44	0.121	NS
Polysaccharides	0.37 $\pm$ 0.20	0.616 $\pm$ 0.05	2.67	< 0.05
Wet weight	314 $\pm$ 72.2	400 $\pm$ 38	2.36	< 0.05

NS - not significant

Table 6.11

Effect of eyestalk ablation on O. platytarsis: changes in composition of CSG of crabs destalked during March/April, at 10 days postoperation and at moulting after destalking (quantity expressed as mg/individual, $\bar{X} \pm SD$)

	10 days after destalkation (n=5)	At moulting after destalka- tion (n=5)	t	p
Moulting stage	C ₃ -C ₄	E		
TCA-precipitable proteins	29.05 $\pm$ 6.1	22.25 $\pm$ 2.26	2.34	< 0.05
Free amino acids (FAA)	1.44 $\pm$ 0.29	1.00 $\pm$ 0.012	3.39	< 0.01
Oligosaccharide fraction	1.76 $\pm$ 0.34	1.43 $\pm$ 0.45	1.31	NS
Polysaccharides	0.37 $\pm$ 0.20	0.477 $\pm$ 0.19	0.868	NS
Wet weight	314 $\pm$ 72.2	253 $\pm$ 59	1.46	NS

NS - not significant

Table 6.12

Effect of eyestalk ablation on O. platytarsis: changes in composition of CSG of crabs destalked during March/April, at 20 days postoperation and at moulting after destalking (quantity expressed as mg/individual, $\bar{X} \pm SD$).

	20 days after destalkation (n=5)	At moulting after destalka- tion (n=5)	t	p
Moulting stage				
TCA-precipitable proteins	D ₂ -D ₃	E		
Free amino acids (FAA)	33.33 $\pm$ 5.06	22.25 $\pm$ 2.26	4.47	< 0.005
Oligosaccharide fraction	0.885 $\pm$ 0.25	1.00 $\pm$ 0.12	1.03	NS
Polysaccharides	1.79 $\pm$ 0.44	1.43 $\pm$ 0.45	1.28	NS
Wet weight	0.616 $\pm$ 0.05	0.477 $\pm$ 0.19	1.58	NS
	400 $\pm$ 38	253 $\pm$ 59	4.69	< 0.002
NS - not significant				

Table 6.13

Effect of eyestalk ablation of O. platytarsis: changes in composition of CSG during September/October, 10 days postoperation (quantity expressed as mg/individual, $\bar{X} \pm \text{SD}$).

	Destalked (n=5)	Controls* (n=5)	t	p
Moulting stage				
TCA-precipitable proteins	C ₄ -D ₀ 24.41 $\pm$ 4.7	C ₄ 21.25 $\pm$ 3.6	1.57	NS
Free amino acids (FAA)	0.534 $\pm$ 0.13	0.297 $\pm$ 0.035	3.94	< 0.005
Oligosaccharide fraction	0.991 $\pm$ 0.34	1.14 $\pm$ 0.26	0.779	NS
Polysaccharides	0.20 $\pm$ 0.07	0.246 $\pm$ 0.008	1.46	NS
Wet weight	246.64 $\pm$ 56.97	216 $\pm$ 77.08	0.715	NS

* group-III

NS - not significant

Table 6.14

Effect of eyestalk ablation on O. platytarsis: changes in composition of CSG during September/October, 20 days postoperation (quantity expressed as mg/individual, $\bar{X} \pm \text{SD}$).

	Destalked (n=5)	Controls* (n=5)	t	p
Moulting stage	D ₀ -D ₁	C ₄		
TCA-precipitable proteins	30.04 $\pm$ 4.59	22.28 $\pm$ 3.5	3.00	< 0.02
Free amino acids (FAA)	0.418 $\pm$ 0.07	0.216 $\pm$ 0.03	5.94	< 0.001
Oligosaccharide fraction	1.02 $\pm$ 0.16	1.00 $\pm$ 0.21	0.169	NS
Polysaccharides	0.25 $\pm$ 0.05	0.273 $\pm$ 0.162	0.304	NS
Wet weight	280.8 $\pm$ 40.41	220 $\pm$ 62.5	1.83	NS

* group-III
NS - not significant

Table 6.15

Effect of eyestalk ablation on O. platytarsis: changes in composition of CSG during September/October, at moulting after destalking (quantity expressed as mg/individual $\bar{X} \pm$ SD).

	Destalked (n=5)	Controls* (n=5)	t	p
Moulting stage	E	C ₄		
TCA-precipitable proteins	29.39 $\pm$ 3.24	22.66 $\pm$ 2.93	3.45	< 0.01
Free amino acids (FAA)	1.54 $\pm$ 0.43	0.291 $\pm$ 0.062	6.43	< 0.001
Oligosaccharide fraction	1.53 $\pm$ 0.75	1.29 $\pm$ 0.061	0.714	NS
Polysaccharides	0.52 $\pm$ 0.32	0.208 $\pm$ 0.013	2.18	NS
Wet weight	274.33 $\pm$ 70.74	214.6 $\pm$ 84.4	1.21	NS
* group-III				
NS - not significant				

Table 6.16

Effect of eyestalk ablation on O. platytarsis: changes in composition of CSG of crabs destalked at 10 days and 20days postoperation during September/October (quantity expressed as mg/individual, $\bar{X} \pm \text{SD}$).

	10 days postoperation (n=5)	20 days postoperation (n=5)	t	p
Moulting stage				
TCA-precipitable proteins	C_4-D_0 25.41 ± 4.70	D_0-D_1 30.04 ± 4.59	1.58	NS
Free amino acids (FAA)	0.053 ± 0.13	0.418 ± 0.07	1.76	NS
Oligosaccharide fraction	0.991 ± 0.34	1.02 ± 0.16	0.173	NS
Polysaccharides	0.20 ± 0.07	0.25 ± 0.05	1.30	NS
Wet weight	246 ± 56.97	280.8 ± 40.41	1.09	NS

NS - not significant

Table 6.17

Effect of eyestalk ablation on O. platytarsis: changes in composition of CSG of crabs destalked during September/October, at 10 days postoperation and at moulting after destalking (quantity expressed as mg/individual, $\bar{X} \pm SD$).

	10 days postoperation (n=5)	At moulting (n=5)	t	p
Moulting stage	C ₄ -D ₀	E		
TCA-precipitable proteins	25.41 $\pm$ 4.7	29.39 $\pm$ 3.24	1.56	NS
Free amino acids (FAA)	0.534 $\pm$ 0.13	1.54 $\pm$ 0.43	5.01	< 0.002
Oligosaccharide fraction	0.991 $\pm$ 0.34	1.53 $\pm$ 0.75	0.465	NS
Polysaccharides	0.20 $\pm$ 0.07	0.52 $\pm$ 0.32	2.19	NS
Wet weight	246.64 $\pm$ 56.97	274.33 $\pm$ 70.74	0.682	NS
NS - not significant				

Table 6.18
Effect of eyestalk ablation on O. platytarsis: changes in composition of CSG of crabs during September/October, 20 days postoperation and at moulting ^{after} destalking (quantity expressed as mg/individual, $\bar{X} \pm \text{SD}$).

Moulting stage	20 days postoperation (n=5)		t	p
	D ₀ -D ₁	At moulting (n=5)		
TCA-precipitable proteins	30.04 $\pm$ 4.59	29.39 $\pm$ 3.24	0.259	NS
Free amino acids (FAA)	0.418 $\pm$ 0.07	1.54 $\pm$ 0.43	5.76	< 0.001
Oligosaccharide fraction	1.02 $\pm$ 0.16	1.53 $\pm$ 0.75	1.49	NS
Polysaccharides	0.25 $\pm$ 0.05	0.52 $\pm$ 0.32	1.87	NS
Wet weight	280.8 $\pm$ 40.41	274.33 $\pm$ 70.74	0.178	NS

NS - not significant

Table 6.19

Effect of eyestalk ablation on O. platytarsis: changes in composition of the vas deferens during September/October, 10 days postoperation (quantity expressed as mg/individual, $\bar{X} \pm SD$).

	Destalked (n=5)	Controls* (n=5)	t	p
Moulting stage				
TCA-precipitable proteins	C4-D ₀ 25.26 $\pm$ 3.7	C4 26.71 $\pm$ 4.7	0.542	NS
Free amino acids (FAA)	0.316 $\pm$ 0.053	0.217 $\pm$ 0.04	3.34	< 0.02
Oligosaccharide fraction	0.669 $\pm$ 0.14	0.694 $\pm$ 0.24	0.201	NS
Polysaccharides	0.529 $\pm$ 0.06	0.63 $\pm$ 0.12	1.68	NS
Wet weight	251 $\pm$ 60.24	204 $\pm$ 83.64	1.02	NS

* group-III

NS - not significant

Table 6.20

Effect of eyestalk ablation on O. platytarsis: changes in composition of the vas deferens during September/October, 20 days postoperation (quantity expressed as mg/individual, $\bar{X} \pm SD$).

	Destalked (n=5)	Controls* (n=5)	t	p
Moulting stage	D ₀ -D ₁	C ₄		
TCA-precipitable proteins	28.07 $\pm$ 4.33	25.18 $\pm$ 3.52	0.044	NS
Free amino acids (FAA)	1.057 $\pm$ 0.116	0.201 $\pm$ 0.062	14.56	< 0.001
Oligosaccharide fraction	0.867 $\pm$ 0.211	0.688 $\pm$ 0.055	1.84	NS
Polysaccharides	0.894 $\pm$ 0.61	0.874 $\pm$ 0.046	0.073	NS
Wet weight	200 $\pm$ 42.17	211.5 $\pm$ 91.54	0.255	NS

* group-III
NS - not significant

Table 6.21

Effect of eyestalk ablation on O. platytarsis: changes in composition of the vas deferens during September/October, at moulting after destalking (quantity expressed as mg/individual, $\bar{X} \pm SD$).

	Destalked (n=5)	Controls* (n=5)	t	p
Moulting stage	E	C ₄		
TCA-precipitable proteins	30.79 $\pm$ 3.41	25.58 $\pm$ 2.85	2.62	< 0.05
Free amino acids (FAA)	0.677 $\pm$ 0.371	0.24 $\pm$ 0.036	2.62	< 0.05
Oligosaccharide fraction	0.991 $\pm$ 0.292	0.607 $\pm$ 0.31	2.01	NS
Polysaccharides	0.614 $\pm$ 0.073	0.746 $\pm$ 0.21	1.34	NS
Wet weight	236.4 $\pm$ 103.5	207.6 $\pm$ 79.54	0.494	NS

* group III

NS - not significant.

Table 6.22

Effect of eyestalk ablation on O. platytarsis: changes in composition of the vas deferens of crabs destalked during September/October, at 10 and 20 days postoperation (quantity expressed as mg/individual, $\bar{X} \pm \text{SD}$).

	10 days postoperation (n=5)	20 days postoperation (n=5)	t	p
Moulting stage				
TCA-precipitable proteins	C_4-D_0	D_0-D_1		
Free amino acids (FAA)	25.26 ± 3.7	28.07 ± 4.33	1.10	NS
Oligosaccharide fraction	0.316 ± 0.053	1.057 ± 0.116	13.00	< 0.001
Polysaccharides	0.669 ± 0.14	0.867 ± 0.211	1.75	NS
Wet weight	0.529 ± 0.06	0.899 ± 0.61	1.33	NS
	251 ± 60.24	200 ± 42.17	1.55	NS

NS - not significant

Table 6.23

Effect of eyestalk ablation on O. platytarsis: changes in composition of the vas deferens of crabs destalked during September/October, at 20 days postoperation and at moulting after destalking (quantity expressed as mg/individual, $\bar{X} \pm SD$).

	10 days postoperation (n=5)	At moulting (n=5)	t	p
Moulting stage				
TCA-precipitable proteins	C_4-D_o	E		
Free amino acids (FAA)	25.26 ± 3.70	30.79 ± 3.41	2.46	< 0.05
Uligosaccharide fraction	0.316 ± 0.053	0.677 ± 0.371	2.16	NS
Polysaccharides	0.669 ± 0.14	0.991 ± 0.292	2.23	NS
Wet weight	0.529 ± 0.06	0.614 ± 0.073	2.01	NS
	251 ± 60.24	236.4 ± 103.5	0.273	NS
NS - not significant				

Table 6.24

Effect of eyestalk ablation on O. platytarsis: changes in composition of the vas deferens of crabs destalked during September/October, at 20 days postoperation and at moulting after destalking (quantity expressed as mg/individual, $\bar{X} \pm SD$).

	20 days postoperation (n=5)	At moulting (n=5)	t	p
Moulting stage	D ₀ -D ₁	E		
TCA-precipitable proteins	28.07 $\pm$ 4.33	30.79 $\pm$ 3.41	1.10	NS
Free amino acids (FAA)	1.057 $\pm$ 0.116	0.667 $\pm$ 0.371	2.19	NS
Oligosaccharide fraction	0.867 $\pm$ 0.211	0.991 $\pm$ 0.292	0.77	NS
Polysaccharides	0.894 $\pm$ 0.61	0.61 $\pm$ 0.073	1.02	NS
Wet weight	200 $\pm$ 42.17	236.4 $\pm$ 103.5	0.729	NS
NS - not significant				

Table 6.25

Table showing the components of the vas deferens in destalked+eyestalk-extract injected and normal O. platytarsis during September/October, 10 days postoperation (quantity expressed as mg/individual, $\bar{X} \pm$ SD).

	Destalked** eyestalk-extract injected (n=5)	Normal* (n=5)	t	p
Moulting stage	C ₄	C ₄		
TCA-precipitable proteins	26.01 $\pm$ 4.7	26.71 $\pm$ 4.7	0.236	NS
Free amino acids (FAA)	0.164 $\pm$ 0.14	0.217 $\pm$ 0.08	0.736	NS
Uligosaccharide fraction	0.547 $\pm$ 0.16	0.694 $\pm$ 0.29	0.993	NS
Polysaccharides	0.529 $\pm$ 0.24	0.63 $\pm$ 0.12	0.842	NS
Wet weight	218 $\pm$ 93.52	204 $\pm$ 83.64	0.249	NS

** group-II

* group-III

NS - not significant.

Table 6.26

Table showing the components of the vas deferens in destalked#eyestalk-extract injected and normal O. platytarsis during September/October, 20 days postoperation (quantity expressed as mg/individual, $\bar{X} \pm$ SD).

	Destalked+** eyestalk-extract injected (n=5)	Normal* (n=5)	t	p
Moulting stage	C ₄			
TCA-precipitable proteins	27.7 $\pm$ 5.2	28.18 $\pm$ 3.52	0.166	NS
Free amino acids (FAA)	0.374 $\pm$ 0.175	0.201 $\pm$ 0.062	2.08	NS
Oligosaccharide fraction	0.625 $\pm$ 0.04	0.688 $\pm$ 0.055	1.91	NS
Polysaccharides	0.767 $\pm$ 0.173	0.874 $\pm$ 0.046	1.35	NS
Wet weight	216 $\pm$ 85.44	211.5 $\pm$ 91.54	0.075	NS

** group-II

* group-III

NS - not significant.

Table 6.27

Table showing the components of the CSG in destalked+eyestalk-extract injected and normal O. platytarsis during September/October, 10 days postoperation (quantity expressed as mg/individual, $\bar{X} \pm SD$).

	Destalked** eyestalk -extract injected (n=5)		Normal* (n=5)	t	p
Moulting stage					
TCA-precipitable proteins	C ₄				
Free amino acids (FAA)	24.39 $\pm$ 4.6		21.25 $\pm$ 3.6	1.20	NS
Oligosaccharide fraction	0.20 $\pm$ 0.21		0.297 $\pm$ 0.035	0.925	NS
Polysaccharides	1.07 $\pm$ 0.212		1.14 $\pm$ 0.26	0.467	NS
Wet weight	0.248 $\pm$ 0.10		0.246 $\pm$ 0.008	0.045	NS
	215.25 $\pm$ 68.58		216 $\pm$ 77.08	0.016	NS

** group-II

* group-III

NS- not significant

Table 6.28

Table showing the components of the CSG in destalked+eyestalk-extract injected and normal O. platytarsis during September/October, 20 days postoperation (quantity expressed as mg/individual, $\bar{X} \pm$ SD).

	Destalked+** eyestalk-extract injected (n=5)	Normal* (n=5)	t	p
Moulting stage	C ₄	C ₄		
TCA-precipitate proteins	23.02 $\pm$ 2.5	22.25 $\pm$ 3.5	0.354	NS
Free amino acids (FAA)	0.534 $\pm$ 0.48	0.216 $\pm$ 0.03	1.51	NS
Oligosaccharide fraction	1.42 $\pm$ 0.329	1.00 $\pm$ 0.21	0.668	NS
Polysaccharides	0.203 $\pm$ 0.036	0.273 $\pm$ 0.162	0.837	NS
Wet weight	253.6 $\pm$ 56.27	220 $\pm$ 62.5	0.836	NS
** group-II				
* group-III				
NS - not significant				

Table 6.29

Table showing the components of the vas deferens of destalked (September/October) and normal Q. platytarsis during premoult (quantity expressed as mg/individual, $\bar{X} \pm SD$).

	Destalked (n=5)	Normal (with eyestalks) (n=4)	t	p
Moulting stage	D ₀ -D ₁	D ₀ -D ₂		
TCA-precipitable proteins	28.07 $\pm$ 4.33	23.04 $\pm$ 6.86	1.35	NS
Free amino acids (FAA)	1.057 $\pm$ 0.116	0.244 $\pm$ 0.122	10.22	< 0.001
Oligosaccharide fraction	0.867 $\pm$ 0.211	0.639 $\pm$ 0.137	1.86	NS
Polysaccharides	0.894 $\pm$ 0.61	1.25 $\pm$ 0.503	0.936	NS
Wet weight	200 $\pm$ 42.17	223 $\pm$ 40.08	0.830	NS

NS - not significant

	Destalked (n=5)	Normal (with eyestalks) (n=4)	t	p
Moulting stage	D ₂ -D ₃	D ₀ -D ₂		
TCA-precipitable proteins	31.46 ± 6.53	23.04 ± 6.86	1.88	NS
Free amino acids (FAA)	0.85 ± 0.44	0.244 ± 0.122	2.64	< 0.05
Oligosaccharide fraction	0.708 ± 0.092	0.639 ± 0.137	0.906	NS
Polysaccharides	0.841 ± 0.39	1.25 ± 0.503	1.38	NS
Wet weight	276 ± 84	223 ± 40.08	1.15	NS

NS - not significant

Table 6.31

Table showing the components of the CSG of destalked (September/October) and normal O. platytarsis during premoult (quantity expressed as mg/individual, $\bar{X} \pm \text{SD}$)

	Destalked (n=5)	Normal (with eyestalks) (n=4)	t	p
Moulting stage	D _o -D ₁	D _o -D ₂		
TCA-precipitable proteins	30.04 $\pm$ 4.59	27.36 $\pm$ 0.918	1.13	NS
Free amino acids (FAA)	0.418 $\pm$ 0.07	0.226 $\pm$ 0.083	3.77	< 0.01
Oligosaccharide fraction	1.02 $\pm$ 0.16	1.62 $\pm$ 0.569	2.28	NS
Polysaccharides	0.15 $\pm$ 0.05	0.878 $\pm$ 0.554	2.98	< 0.05
Wet weight	280.8 $\pm$ 40.41	278 $\pm$ 44.89	0.098	NS

NS - not significant

	Destalked (n=5)	Normal (with eyestalks) (n=4)	t	p
Moulting stage	D ₂ -D ₃	D ₀ -D ₂		
TCA-precipitable proteins	33.33 ± 5.06	27.36 ± 0.918	2.297	NS
Free amino acids (FAA)	0.885 ± 0.25	0.226 ± 0.083	4.99	< 0.002
Oligosaccharide fraction	1.79 ± 0.44	1.62 ± 0.569	0.507	NS
Polysaccharides	0.616 ± 0.05	0.878 ± 0.554	1.071	NS
Wet weight	400 ± 38	278 ± 44.89	4.42	< 0.005
NS - not significant				

	AVD-I	AVD-II	AVD-III	MVD-I	MVD-II	ED	CSG
Eyestalkless	29.4±3.63	33.25±13.3	9.6±4.3	6.2±3.1	49.9±3.3	39.3±11.9	25.75±15.74
Eyestalks intact	2.31±8.27	17.7±7.3	6.5±2.35	5.2±4.06	50.4±4.3	12.6±2.1	9.75±4.9
t	1.35	4.55	2.84	0.55	0.26	3.7	4.34
p	> 0.2	< 0.001	< 0.01	> 0.5	> 0.5	< 0.02	< 0.001

AVD - anterior vas deferens; MVD-mid vas deferens; ED- ejaculatory duct;
 CSG - coral-shaped accessory gland.

6.4. DISCUSSION

Experiments involving destalking, reported in this chapter, clearly show that in male O. platytarsis eyestalk principles (GIH?) directly or indirectly (via androgenic glands?) restrain the degree of activity of the epithelial cells and the quantity of various biochemical components in the vas deferens and CSG. Changes in composition of the vas deferens and CSG due to destalking could be restored by injection of eyestalk extract (Tables 6.25 to 6.28), suggesting that hormonal principles of eyestalk origin are possibly involved. Following destalking, the wall cells of CSG showed a significant increase in height and nuclear size. Twenty days after eyestalk ablation, there was a significant increase in TCA-precipitable proteins of CSG of O. platytarsis which indicates that the protein synthetic machinery of CSG may be under the inhibitory control of eyestalk principles. Endocrine control of male accessory reproductive glands is well documented among arthropods in insects (Gillott, 1985). In monarch butterflies, extra-production of juvenile hormone results in post-eclosive enlargement of the accessory gland. Conceivably, the protein secretion of the accessory gland is influenced by juvenile hormone (Herman, 1975). In male Rhodnius prolixus, accessory sex glands produce a number of proteins

which are pressed into service to protect gametes and to facilitate their transfer into the female system. The co-ordination of the synthesis of these proteins with reproductive activity is expected to be under the neuroendocrine system (Barker and Davey, 1981). Evidence for an inhibitory control by neurosecretory cells of the optic tentacles on activity of the albumen glands has been reported in snails: in Cryptozona semirugata, for instance, ablation of the optic tentacles resulted in an increase in the albumen gland index within a couple of weeks (Kulkarni and Utkar, 1981). On the contrary, in Cryptozona belangeri continued supply of the optic tentacular principles is necessary to sustain activity of the male accessory reproductive organs (dart sac) (Sriramulu and Magdalene, 1981).

The male reproductive system of O. platytarsis responded unevenly to eyestalk ablation experiments conducted during the two different seasons. Not only did the magnitude of response elicited by the destalking on the male physiology vary during September/October and March/April, but the effects of the deprivation of eyestalk principles on different parts of the male reproductive system too were not the same. Bilateral eyestalk ablation, conducted on crabs in their intermoult (stage C₄) during September/October, when the androgenic

gland cells were apparently inactive, resulted in less spectacular changes. At 10 days postoperation, except for the increase in FAA, biochemical components and wet weight of the CSG and vas deferens of experimental animals were comparable to those of the controls (Table 6.13 and 6.19). There were no conspicuous changes in the histology of vas deferens or CSG either. At 20 days postoperation, when all the experimental animals had reached premoult (stage D_0 - D_1), there was a significant rise in total protein content of the CSG, but the wet weight did not increase over this period significantly (Table 6.14). Even when the destalked crabs reached stage E (27 to 35 days postoperation), the wet weight of the CSG did not show appreciable changes (Table 6.15). In the vas deferens, eyestalk ablation did not induce any conspicuous change either in wet weight or in quantitative profile of nearly all (except FAA) biochemical components, when assayed 20 days postoperation; the crabs had by this time reached stage D_0 - D_1 of the moulting cycle. Contents of protein and carbohydrate and the wet weight of CSG of experimental animals, 20 days postoperation, were comparable to those of controls and to those of experimental crabs at 10 days postoperation (Tables 6.20 and 6.22).

The incidence of nuclear pycnosis in epithelial

cells (and disorganization of cells) of AVD of eyestalkless individuals (notwithstanding the increase in total cell size) is possibly indicative of cellular exhaustion setting in after the initial phase of hyperactivity caused by withdrawal of the inhibitory eyestalk principles, possibly GIH. Despite the apparent hypertrophy of the secretory epithelium of AVD, bilateral eyestalk ablation (even after 20 days) in O. platytarsis did not lead to a concomitant increase in protein content of the vas deferens, which is intriguing because protein constitutes the chief component of the primary semen of this species. At the time of moulting after destalking, though protein content of vas deferens did increase significantly in comparison to controls, the wet weight of the vas deferens and its carbohydrate content did not show any significant change (Table 6.21). Apparently, the withdrawal of eyestalk principles during September/October produced only a slow response in the male sex duct and accessory reproductive gland of O. platytarsis. Such season-related differences in responses of tissues to bilateral eyestalk ablation are well known in the crab Paratelphusa hydrodromous (see Kurup and R.G. Adiyodi, 1984). In female P. hydrodromous, the acceleration of ovarian growth induced by bilateral eyestalk ablation during the somatic phase, when the physiology is biased in favour

of somatic growth, is only of a smaller magnitude than that brought about by the same operation during the reproductive phase. In male P. hydrodromous, somatic growth and reproduction are kept under check by effective release of eyestalk principles (MIH and GIH) during the somatic phase of the intermoult. Male genital system responds to bilateral eyestalk ablation even during the somatic phase in P. hydrodromous. Total protein, FAA and carbohydrate contents of the testis, vas deferens and semen of male P. hydrodromous, destalked during the somatic phase, registered a significant rise at 10 days postoperation and there was also a hypertrophy of the ductal epithelial cells (Mathad and K.G. Adiyodi, 1981; Mathad, 1983).

In O. platytarsis, withdrawal of eyestalk principles during March/April, when the androgenic glands were large with healthy cells, resulted in a significant rise in total wet weight of the vas deferens (Table 6.1) and in an insignificant rise in that of the CSG (Table 6.7), by 10 days postoperation. In the vas deferens, there was concomitantly a significant increase in total TCA-precipitable protein and oligosaccharide fraction, but the rise in levels of FAA and polysaccharides were not significant (Table 6.1). At 10 days postoperation the rise in total TCA-precipitable proteins and polysaccharides of CSG was statistically insignificant

but the rise in FAA pool and oligosaccharide fraction was significant (Table 6.7). A further rise in total wet weight of the CSG (Table 6.8) and vas deferens (Table 6.2) was observed at 20 days postoperation; by this time all the destalked males had reached stages D_2 - D_3 of the moulting cycle. TCA-precipitable protein content of the CSG (Table 6.8) and vas deferens (Table 6.2) had by 20 days postoperation reached values significantly higher than those of the controls. A similar rise was also observed in total carbohydrate (oligosaccharide fraction + polysaccharides) content of the CSG (Table 6.8). By 22-25 days postoperation, all destalked crabs had moulted (stage E); CSG assayed at this time revealed a remarkable fall in wet weight and also in total proteins (Table 6.12). The vas deferens, on the other hand, continued to gain appreciably both in total wet weight and in content of total proteins (Table 6.3). During March/April, by 20 days postoperation, the height of the epithelial cells of AVD-II was more than that of the controls. The nuclei were large with distinct chromatin blocking and the cytoplasm was vacuolated, suggesting that the cells were in active synthetic phase. As crabs destalked during March/April entered stage E (22-23 days postoperation), their vas deferens presented a histological picture reminiscent of that of crabs at 20

days after destalking during September/October. The only obvious difference was that pycnotic nuclei were fewer in crabs destalked during March/April.

Comparison of results of the two series of identical experiments conducted during two different seasons of the year thus reveals that the restraining influence exerted by eyestalks on activity of the male reproductive system in O. platytarsis is more intense during March/April than during September/October. In the fresh water crab P. hydrodromous also, the eyestalks exert an inhibitory influence on activity of the male reproductive system during the reproductively active season (i.e. August/September). During this period increase in height and secretory activity of the cells of vas deferens was evident by 10 days after destalking; there was also an increase in wet weight and biochemical components of the vas deferens. Interestingly, in P. hydrodromous, the degree of initial hypertrophy of the epithelial cells of the vas deferens induced by destalking during the somatic phase of the intermoult is comparable to that induced by destalking during the reproductive phase of the intermoult (see Mathad, 1983 for details).

Our observations show that bilateral eyestalk ablation during March/April results in production of

a large number of degenerated cells in androgenic glands of O. platytarsis. During September/October, when the androgenic gland is already in regressed condition, destalkation fails to revive androgenic gland activity to any cytologically detectable level, even by 20 days postoperation. It may be recalled that in the protandric shrimp Pandalus platyceros eyestalk inhibition on androgenic glands is seasonal and is stronger in winter than in summer (Hoffman, 1968). In Parapenaeopsis hardwickii too, androgenic gland activity is regulated by the eyestalk principles (Kulkarni et al., 1984). The appearance of a large number of degenerated cells in the androgenic gland following eyestalk ablation during March/April supports, indirectly though, the holocrine nature of the secretion of this gland in O. platytarsis (as suspected earlier - see Chapter V). The ready response of the male genital system to eyestalk ablation during March/April which is manifested as increased activity suggests that inhibitory principle/s (GIH?) contained in eyestalks may normally suppress secretion of the androgenic hormone. The effect of deprivation of eyestalk principles appears to be all the more pronounced when the androgenic gland cells are in a state to synthesize more androgenic hormone.

The year-round analyses of the vas deferens factor (Vdf) and gland (CSG) factor (Glf) and of the percentage of

protein in vas deferens and CSG of C. platytarsis (see Chapter V), have shown that after reaching a peak in August, these values decrease from September. Eyestalk ablation during September/October produces only a marginal impact on activity of the male reproductive system, because the androgenic gland is already in a regressed condition and little further increase in secretion of the androgenic hormone is possible during the season. It may be also argued that the GIH level being already low, the androgenic hormone has been completely released during September/October and that the total withdrawal of the restraining principles from the eyestalks therefore produces no effect on the reproductive system. In P. hydrodromous, eyestalk ablation does not lead to any remarkable change in histology of the androgenic glands even after 30 days during the reproductively active season (Mathad, 1983).

Results of eyestalk ablation experiments conducted by us during March/April reveal that the increase in wet weight of CSG and vas deferens of O. platytarsis is usually accompanied by an increase in various biochemical components, which in turn is a reflection of the increased synthetic activity following destalkation. In the freshwater crab P. hydrodromous also, destalkation leads to an increase in total wet weight and quantities of various

biochemical components of the vas deferens (Mathad, 1983). A synchronous variation in wet weight of the collateral gland and its protein content in relation to reproductive cycle has been reported in the American cockroach Periplaneta americana (Iris and Sin, 1984). When destalked O. platytarsis entered stage E, CSG registered a significant decrease in wet weight and in total protein content (Table 6.12). Such decreases in total wet weight and in quantities of various biochemical components of the reproductive system, after reaching peak values in the initial stage, are not uncommon among decapods. Again in P. hydrodromous, a reduction (as judged at 30 days postoperation) in wet weight and in quantities of major biochemical components of the vas deferens has been observed following primary increase (at 10 days postoperation) after destalkation; this is believed to be due to resorption of the seminal components. The reduction in quantities of various biochemical components of vas deferens which occurs under conditions of prolonged destalked state is considered to be abnormal, and it is suggested that for the normal functioning of the male reproductive system availability in circulation of a certain threshold titre of GIH is necessary (Mathad and K.G. Adiyodi, 1981; Mathad, 1983).

When CSG is implanted into female O. platytarsis

and cultured in vivo for 10 days the normal shape of the secretory tubules is not visible; its cells become indistinct and the fibrous appearance of the secretion is lost. On the contrary, CSG tubules cultured in vivo in normal male crabs have a morphology and histology fairly similar to those of normal glands. Results of in vivo culture experiments suggest that the CSG of O. platytarsis requires a blood-borne factor present only in males, probably the androgenic hormone, for normal maintenance of the fibrous nature of its secretory products. In crustaceans, androgenic hormone is known to be essential for the normal maintenance and functioning of the male reproductive organs (Charniaux-Cotton, 1960). CSG of O. platytarsis appears to be thus influenced not only by eyestalk hormones (possibly GIH), but also by a male-specific blood-borne factor (possibly the androgenic hormone). Interestingly, in pulmonate gastropods, 'the transplantation of juvenile gonads or reproductive tracts into the haemocoel of normal or castrated adults shows that the albumen gland and the hermaphroditic ducts are controlled by the gonad via a 'blood-borne factor' (Sriramulu, 1982).

Variations in levels of free amino acids (FAA) in vas deferens and CSG of destalked O. platytarsis are noteworthy (Tables 6.1 to 6.3 and 6.13 to 6.15). During September/October, destalkation leads to increase in FAA pool of both CSG and vas deferens even by 10 days

postoperation, and the quantity of FAA is kept high until moulting. It may be recalled that the wet weights of the vas deferens and CSG of destalked crabs vary only marginally from those of normal animals. On the contrary, during March/April, quantities of most of the biochemical components and wet weight of the vas deferens undergo significant increase by 10 days postoperation, but no such trend is shown by FAA. In CSG, though there is an initial increase in FAA level after destalkation during March/April, the value decreases significantly by 20 days postoperation. Our observations described earlier in Chapter V have shown that seasonal variations in FAA in the reproductive system of O. platytarsis are indeed unique. Studies on free amino acid pool in male accessory reproductive glands have revealed that significant alterations in quantity of FAA may occur in insects before and after mating; these alterations appear to be related to the utilization of the secretory product of the gland for the formation of the spermatophore or for the formation of the seminal plasma. Further, it is considered that in insects at least a few amino acids (e.g., aspartate and glutamate) are synthesized from glucose which in turn can be used for the elaboration of other amino acids or for the production of proteinaceous seminal fluid (Ranganathan, 1982).

A comparison of experimental crabs that had been precociously initiated into premoult with normal crabs that had naturally entered premoult (obtained during different months) shows that the different biochemical components as well as wet weight of vas deferens and CSG are almost comparable (Table 6.29, 6.30 and 6.31). This observation seems to indirectly indicate that the changes in reproductive system of O. platytarsis due to eyestalk ablation run more or less synchronously with the changes in moulting stage. This appears to be the case in normal crabs also with their eyestalks intact. Nevertheless, during March/April the total wet weight of the vas deferens and its protein content did increase (Table 6.1) significantly in the intermoult stage itself in destalked crabs in comparison to controls. Similarly, the total wet weight of CSG of the destalked crabs in premoult during March/April was significantly higher than that of normal premoult crabs (Table 6.32). These observations possibly suggest that the enhanced production of various seminal components in male reproductive system of O. platytarsis occurs at a greater pace under altered experimental conditions. Moult-related changes have been reported in semen production in the freshwater shrimp, Macrobrachium idella: the ductal cells of vas deferens are at the height of their synthetic activity

during intermoult, but maximum secretion in the lumen of the sperm duct is observed during late premoult (Sreekumar and R.G. Adiyodi, 1981; Sreekumar, 1985).

Since artificial insemination of female O. platytarsis with the semen of destalked animals has not been attempted, one can at this stage only speculate about the quality of semen produced under experimental conditions. Comparison of the individual components of the vas deferens or CSG of destalked animals with those of the controls shows that the changes precipitated by eyestalk removal in various biochemical components are not uniform. Eyestalk ablation seems to affect the male and female reproductive physiology slightly differently. For instance, eyestalk ablation in female Paratelphusa hydrodromous leads to abnormal increase in production of yolk which is chemically impoverished (Anilkumar and K.G. Adiyodi, 1980), but the composition of semen produced by destalked male P. hydrodromous is comparable to that produced by controls (Mathad, 1983).

6.5. SUMMARY

1. Two identical series of experiments involving eyestalk ablation were conducted during two different seasons on male O. platytarsis, one during March/April (androgenic glands active) and the other

during September/October (androgenic glands inactive). Histological and biochemical analyses have shown that eyestalk principle/s directly or indirectly restrain the activity of the sperm duct and CSG of this species.

2. The magnitude of response elicited by eyestalk ablation on male physiology varied during September/October and March/April; the effects of deprivation of eyestalk principle/s on different parts of the male reproductive system also were different during the two seasons.
3. Bilateral eyestalk ablation conducted on male O. platytarsis in their intermoult (stage C₄) during September/October resulted in less spectacular changes. Biochemical or cytological changes appeared in the vas deferens and CSG only by 20 days post-operation, when the crabs entered stage D₀-D₁ of the moulting cycle.
4. During September/October, at 20 days postoperation, there was a significant rise in TCA-precipitable proteins of CSG followed by a revival of activity of the secretory epithelium lining the CSG tubules. The increased level^{of} protein content of the CSG observed in destalked crabs continued until they

moulted. Throughout the course of the experiment during this season, the wet weight and carbohydrate (oligosaccharide fraction + polysaccharides) content of the CSG showed little variation in destalked crabs.

5. By 20 days postoperation, during September/October, vas deferens of destalked O. platytarsis presented cytological features different from those of the controls. For instance, in AVD-II, surface of the epithelium of destalked crabs became very uneven and presented a characteristic folded appearance; cytoplasm became vacuolated and pycnotic nuclei were common. Protein content of the vas deferens did not, however, show appreciable difference between experimental and control animals.
6. In vas deferens, during September/October, there was a significant increase in TCA-precipitable protein in destalked crabs as they moulted, but total wet weight and carbohydrate content of the vas deferens showed little variation in destalked crabs.
7. During March/April, the wet weight and total protein and oligosaccharide fraction of the vas deferens of destalked O. platytarsis increased significantly

- early (even by 10 days postoperation) and this increase was maintained until moulting.
8. During March/April, by 20 days postoperation, CSG of destalked crabs registered a significant increase in wet weight, TCA-precipitable proteins and carbohydrates.
 9. The epithelial cells of CSG tubules of destalked crabs, during March/April, became vacuolated as they moulted; by this time CSG had registered a significant fall in wet weight and in TCA-precipitable proteins.
 10. The effect of withdrawal of the eyestalk principle/s on FAA pool of the vas deferens and CSG of O. platytarsis was peculiar.
 11. Destalkation during March/April resulted in production of a large number of degenerated cells in androgenic glands (AG) of O. platytarsis; the same operation during September/October, however, failed to revive AG activity. It is suggested that inhibitory eyestalk principles (GIH?) may normally suppress the holocrine secretion of AG; the effect of deprivation of eyestalk principle/s appears to be more pronounced when AG cells are in a state to synthesize androgenic hormone.

12. The restraining influence exerted by eyestalks on activity of the male reproductive system of O. platytarsis is more intense during March/April than during September/October.
13. When CSG is implanted into female O. platytarsis and cultured in vivo, normal shape of the CSG tubules and of the fibrous secretion is distorted. This suggests that CSG requires a male specific blood-borne factor (probably androgenic hormone) for its normal maintenance.

References

M Sukumaran “Certain aspects of reproductive biology of the ghost crab, *Ocypode Platyt Aris* with special reference to the male” Thesis. Department of Zoology, University of Calicut, 1985

- Adamopoulos, D.A. and Deliyiannis, V. (1983), Seminal plasma magnesium, calcium and inorganic phosphate concentration in normozoospermic and subfertile men. Andrologia, 15, 648-654.
- Adiyodi, K.G. (1982), Some thoughts on the evolution of "reproductive" hormone in invertebrates. In "Progress in Invertebrate Reproduction and Aquaculture" (T. Subramoniam and Sudha Varadarajan, eds.) Indian Society of Invertebrate Reproduction, Madras, pp. 89-93.
- Adiyodi, K.G. and Adiyodi, R.G. (1970), Endocrine control of reproduction in decapod Crustacea. Biol. Rev., 45, 121-165.
- Adiyodi, K.G. and Adiyodi, R.G. (1975), Morphology and cytology of the accessory sex glands in invertebrates. Int. Rev. Cytol., 43, 353-398.
- Adiyodi, K.G. and Adiyodi, R.G. (1976), Seasonal changes in the activity of the androgenic gland of the crab Paratelphusa hydrodromous. Proc. IV All India Symp. Comp. Endocr., Mysore, pp. 1.
- Adiyodi, K.G. and Adiyodi, R.G. (1982), Reproduction vs growth: endocrine programming in the Brachyura (Crustacea: Decapoda). Proc. IXth Int. Sym. Comp. Endocrinol., Hongkong, pp. 238.
- Adiyodi, K.G. and Adiyodi, R.G. (eds.) (1983), "Reproductive Biology of Invertebrates", Vol. II: Spermatogenesis and Sperm Function. John Wiley and Sons Ltd., Chichester.
- Adiyodi, R.G. (1969), Calcium cycle and hepatopancreas in the fresh water crab, Paratelphusa hydrodromous (Herbst). J. Ker. Acad. Biol., 1, 20-27.

- Adiyodi, R.G. (1978), Endocrine control of ovarian function in crustaceans. In "Comparative Endocrinology" (P.J. Gaillard and H.H. Boer, eds.) Elsevier/North Holland, Amsterdam, pp. 25-28.
- Adiyodi, R.G. (1985), Reproduction and its control. In "The Biology of Crustacea" (D.E. Bliss and L.H. Mantel, eds.), Vol. 9, Academic Press, New York, pp. 147-215.
- Adiyodi, R.G. and Adiyodi, K.G. (1970), Lipid metabolism in relation to reproduction and moulting in the crab, Paratelphusa hydrodromous (Herbst). Phospholipids and reproduction. Indian J. Exp. Biol., 8, 222-223.
- Adiyodi, R.G. and Adiyodi, K.G. (1971), Lipid metabolism in relation to reproduction and moulting in the crab, Paratelphusa hydrodromous (Herbst). Cholesterol and unsaturated fatty acids. Indian J. Exp. Biol., 9, 514-515.
- Adiyodi, R.G. and Adiyodi, K.G. (1972), Hepatopancreas of Paratelphusa hydrodromous (Herbst). Histophysiology and the pattern of proteins in relation to reproduction and moult. Biol. Bull., 142, 359-369.
- Adiyodi, R.G. and Adiyodi, K.G. (1974), Ultrastructure of the utriculi majores in mushroom-shaped male accessory gland of Periplaneta americana (L.). Z. Zellforsch., 147, 433-440.
- Adiyodi, R.G. and Subramoniam, T. (1983), Crustacea: In "Reproductive Biology of Invertebrates" Vol. I: Oogenesis (K.G. Adiyodi and R.G. Adiyodi, eds.) John Wiley and Sons, Chichester, pp. 443-489.
- Aiken, D.E. and Waddy, S.L. (1981), Reproductive biology. In "The Biology and Management of Lobsters" Vol. I (Phillips, P. and Cobb, J.S., eds.) Academic Press, 4, 215-276.

- Ajmal Khan S. and Natarajan, R. (1979a), Effect of salinity on the development of the estuarine hermit crab, Clibanarius longitarsus (de Haan). All India Workshop Crust. Reprod. Biol., pp.18.
- Ajmal Khah, S. and Natarajan, R. (1979b), Annual reproductive cycles in five species of hermit crabs from porto novo coast. All India Workshop Crust. Reprod. Biol., pp. 20.
- Amato, G.D. and Payen, G.G. (1978), Mise en évidence du controle endocrine des différentes étapes de la spermatogenèse chez l'ecrevisse Pontastacus leptodactylus leptodactylus (Eschscholtz, 1823) Crustacé, Décapode, Reptantia, Gen. Comp. Endocrinol., 36, 487-496.
- Anderson, W.A. and Ellis, R.A. (1967), Cytodifferentiation of the crayfish spermatozoa: Acrosome formation, transformation of mitochondria and development of microtubules. Zeitschrift für Zellforschung, 77, 80-94.
- Anderson, W.A. and Personne, P. (1970), The localization of glycogen in the spermatozoa of various invertebrate and vertebrate species. J. Cell Biol., 44, 29-51.
- Anilkumar, G. (1980), "Reproductive Physiology of Female Crustaceans", Ph.D. Thesis, Calicut University.
- Anilkumar, G. and Adiyodi, K.G. (1980), Ovarian growth, induced by eyestalk ablation during the prebreeding season, is not normal in the crab, Paratelphusa hydrodromous (Herbst). Int. J. Invert. Reprod., 2, 95-105.
- Arver, S. (1982), Studies on zinc and calcium in human seminal plasma. Acta. Physiol. Scand. Suppl., 0 (507), 1-16.

- Baccetti, B. (1970), "Comparative Spermatology". Academic Press, New York.
- Barker, J.F. and Davey, K.G. (1981), Neuroendocrine regulation of protein accumulation by the transparent accessory reproductive gland of male Rhodnius prolixus. Int. J. Invert. Reprod., 3, 291-296.
- Barnes, H. (1962a), The composition of the seminal plasma of Balanus balanus. J. Exp. Biol., 39, 345-351.
- Barnes, H. (1962b), The oxygen uptake and metabolism of Balanus balanus spermatozoa. J. Exp. Biol., 39, 352-358.
- Barnes, H. (1963), Organic constituents of the seminal plasma of Balanus balanus. J. Exp. Biol., 40, 584-594.
- Barnes, H. and Barnes, M. (1967), The effect of starvation and feeding on the time of production of egg masses in the boreo-arctic cirripede, Balanus balanoides (L). J. Exp. Mar. Biol. Ecol., 1, 1-6.
- Barnes, H. and Blackstock, J. (1974), Biochemical composition of the seminal plasma of the cirripede, Balanus balanus with particular respect to free amino acids and proteins, J. Exp. Mar. Biol. Ecol., 16, 47-85.
- Barnes, H. and Finlayson, D.M. (1962), Presence of ascorbic acid in cirripede semen, Limnol and Oceanogra., 7, 98.
- Batie, R.E. (1983), Rhythmic locomotor activity in the intertidal shore crab Hemigrapsus oregonensis (Brachyura, Gapsidae) from the Oregon Coast (USA). Northwest Sci., 57, 49-56.

- Berreur-Bonnenfant, J. (1967), Action de la glande androgene at du cerveau sur la gametogenese de crustaces Pericardes. Archs. Zool. Exp. Gen., 108, 521-558.
- Berreur-Bonnenfant, J. and Lawrence, F. (1984), Comparative effect of farnesylacetone on macro molecular synthesis in gonads of crustaceans. Gen. Comp. Endocrinol., 54, 462-468.
- Barreur-Bonnenfant, J., Meusy, J.J., Ferezou, J.P., Quesnean-Thierry, A. et Barbier, M. (1973), Recherches sur la sécrétion de la glande androgène des Crustacés Malacostracés: purification d'une substance à activite androgène. C.R. Acad. Sci., 277, 971-974.
- Berry, P.F. (1970), Mating Behaviour, oviposition and fertilization in the spiny lobster Panulirus homarus (Linnaeus). S. African Assoc. Mar. Biol. Res. Ocenogr. Res. Inst. Invest. Rep., 24, 16.
- Best, F. (1906), Z. Wise Mikr., 23, 319, as cited in Pearse (1968).
- Betts, F., Blumenthal, N.C., Posner, A.S., Becker, G.L. and Lehninger, A.L. (1975), Atomic structure of intracellular amorphous calcium phosphate deposits. Proc. Natl. Acad. Sci. USA, 72, 2088-2090.
- Bhargava, P.M. (1957), Nature, Lond., 179, 1120, as cited in Mann (1964).
- Bhargava, P.M., Bishop, M.W.H. and Work, T.S. (1959), Biochem. J., 73, 242, as cited in Mann (1964).

- Black, J.B. (1966), Cyclic male reproductive activities in the dwarf crawfishes Cambarellus shufeldtii (Faxon) and Cambarellus puer (Hobbs). Trans. Am. Microsc. Soc., 85, 214-232.
- Blanchet, M.F., Ozon, R. and Meusy, J.J. (1972), Metabolism of steroids in vitro in the male crab Carcinus maenas Linnae. Comp. Biochem. Physiol., 41, 251-261.
- Bliss, D.E. (1983), "The Biology of Crustacea" Vol. VII, Academic Press, New York and London.
- Bliss, D.E., Van Montfrans, J., Van Montfrans, M. and Boyer, J.R. (1978), Behaviour and growth of the land crab, Gecarcinus lateralis (Fremenville) in South Florida. Bull. American Museum Nat. Hist., 160, 11-152.
- Blum, M.S., Glowska, Z., Taber III, S. (1962), Chemistry of the drone honey bee reproductive system. II. Carbohydrates in the reproductive organs and semen. Ann. Entomol. Soc. Am., 55, 135-139.
- Bomirski, A., Arendarczyk, M., Kawinska, E. and Kleinholz, L.H. (1981), Partial characterization of crustacean gonad~~in~~hibiting hormone. Int. J. Invert. Reprod., 3, 213-219.
- Brasiello, A.R. (1971), Protein synthesis during spermatogenesis of Asellus aquaticus. Experimental Cell Res., 67, 279-288.
- Brendt, K.P. and Prues, W.R. (1979), Accessory glands in reproductive system in male insects. Angew Parasitol., 20, 67-76.
- Brown, G.G. (1966), Ultrastructural studies of sperm morphology and sperm egg interaction in the Decapode Callinectes sapidus. J. Ultrastructure Research, 14, 425-440.

- Brown, G.G. (1970), Some comparative aspects of selected crustacean spermatozoa and crustacean phylogeny. In "Comparative Spermatology" (B. Baccetti, ed.), Academic Press, New York, pp. 183-203.
- Brown, G.G. and Metz, C.B. (1967), Ultrastructural studies on the spermatozoa of two primitive crustaceans Hutchinsoniella macracantha and Derocheilocaris typicus. Z. Zellforsch., 80, 78-92.
- Brown-Woodman, P.D.C. and White, I.G. (1974), Amino acid composition of semen and the secretions of the male reproductive tract. Aust. J. Biol. Sci., 27, 415-422.
- Brushko, Z.K. (1975), Formation des spermatophores chez Astacus leptodactylus. Izvest. Akad. Nauk. Kazakli, S.S.R., U.S.S.R., 13, 31-38.
- Burns, B.G., Sangalang, G.B., Freeman, H.C. and Mcmenemy, M. (1984a), Bioconversion of steroids by the testis of the American lobster, Homarus americanus, in vitro. Gen. Comp. Endocrinol., 54, 422-428.
- Burns, B.G., Sangalang, G.B., Freeman, H.C. and Mcmenemy, M. (1984b), Isolation and identification of testosterone from the serum and testis of the American lobster (Homarus americanus). Gen. Comp. Endocrinol., 54, 429-432.
- Cardin, A. and Meara, M.L. (1953), J. Exp. Biol., 30, 561, as cited in Mann, (1964).
- Carpenter, M.B. and de Roos, R. (1970), Seasonal morphology and histology of the androgenic gland of the crayfish, Orconectes nais. Gen. Comp. Endocrinol., 15, 143-157.
- Chandran, M.R., Subramanian, A. and Thiagarajan, S. (1981),

- Breeding strategy of the shore crab Ocypode rotundatus Meirs to environmental impact at two different localities of the east coast of India. Proc. U.K. Mtg. Int. Soc. Inv. Reprod., New Castle upon Tyne, pp. 17.
- Chandran, M.R., Subramanian, A. and Thiagarajan, S. (1982), Studies on the reproductive and nutritional cycles of two species of fresh water crabs of Cauvery Delta at Thanjavur. In "Progress in Invertebrate Reproduction and Aquaculture" (T. Subramoniam and Sudha Varadarajan eds.) Indian Society of Invertebrate Reproduction, Madras, pp. 123-134.
- Charniaux-Cotton, H. (1954), Découverte chez un Crustacé Amphipode (Orchestia gammarella) d' une glande endocrine responsable de la defferéntiation des caracteres sexuels primaires et secondaires mâles. C.R. Acad. Sci., Paris, 239, 780-782.
- Charniaux-Cotton, H. (1960), Sex determination. In "The physiology of Crustacea", Vol. I (T.H. Waterman, ed.), Academic Press, New York, pp. 411-447.
- Charniaux-Cotton, H. (1970), Sexualité èt activité genitale male chez les Crustacés superieurs, Bull. Soc. Zool., France, 95, 565-594.
- Charniaux-Cotton, H. (1975), Controle hormonal de la defférenciation sexuelle et de l' activite genitale chez les crustaces malacostraces. Tires-a-Part des Publ. Staz. Zool. Napoli., 39 Suppl., 480-509.
- Charniaux-Cotton, H. and Kleinholz, L.H. (1964), Hormones in invertebrates other than insects. In "The Hormones", Vol. IV (G. Pincus, K.V. Thinmann and E.B. Astwood, eds.) Academic Press, New York, pp. 135-198.

- Charniaux-Cotton, H., Payen, G.G. and Ginsburger-Vogel, T. (1985), Crustacea: Sex determination and differentiation. In "Reproductive Biology of Invertebrates" Vol. V. Sexual differentiation and behaviour (K.G. Adiyodi and R.G. Adiyodi, eds.) John Wiley and Sons, U.K., (in press).
- Chayen, J., Bitensky, L. and Butcher, R.G., (1973), "Practical Histochemistry" . John Wiley and Sons, London, New York, Sidney, Toronto.
- Cheung, T.S. (1969), The environmental and hormonal control of growth and reproduction in the adult female stone crab, Menippe mercenaria (Say). Biol. Bull., 136, 327-346.
- Chim, L., Kleinholz, L.H., Payen, G.G. and Laubier-Bonichon, A. (1983), Inhibiting effect of an eyestalk extract from the male prawn, Penaeus vannamei, on the precocious development of the genital apparatus of the eyestalkless male Penaeus japonicus. C.R. Seances Acad. Sci. Ser. III Sci. Vie., 296, 185-190.
- Chinoy, N.J. (1978), Ascorbic acid turnover in animal and human tissues. J. Amin. Morphol. Physiol., Silver Jubilee Volume, pp. 68-85.
- Chinoy, M.R. and Chinoy, N.J. (1984), Histophysiological changes in vas deferens after vasectomy and the prophylactic action of ascorbic acid in mature male albino rats Rattus norvegicus. Monit. Zool. Ital., 18, 89-104.
- Chinoy, N.J. and Seethalakshmi, L. (1977), Biochemical evidence for the occurrence of a possible steroidal mechanism in the reproductive organs of slugs (Laevicaulis alte Ferussac). In "Advances in Invertebrate Reproduction", vol. I (K.G. Adiyodi and R.G. Adiyodi, eds.) Perlam-Kenoth, India, pp. 356-365.

- Chinoy, N.J., Sheth, K.M., Seethalakshmi, L., Parmar, P.Y., Sanjeevan, A.G., Rao, M.V., Sharma, J.D., Chinoy, M.R., Maithili, R.S., Trivedi, D.G., Ashok Kumar, R. and Geetha Ranga, M. (1982), Studies on reproductive physiology of animals with special reference to fertility control. Comp. Physiol. Ecol., 7, 215-251.
- Clark, W.H. (Jr.), Kleve, M.G. and Yudin, A.I. (1981), An acrosome reaction in Natantian sperm. J. Experimental Zool., 218, 279-291.
- Corbel, M.J. and Wood, W.A. (1975), The effect of ascorbic acid depletion on the immune response of the guinea-pig to Brucella abortus strain 19 vaccine. J. Biol. Stand., 3, 167-170.
- Cronin, L.E. (1947), Anatomy and Histology of the male reproductive system of Callinectes sapidus Rathbun. J. Morph., 81, 209-240.
- Datta, S.P. and Ottaway, J.H. (1969), "Biochemistry", Bailliere, Tindall and Cassel, Ltd., London.
- Daudu, C.S., Igboeli, G. and Orji, B.I. (1983), Ejaculate and seminal plasma characteristics of the Yankasa ram. Trop. Vet., 1, 29-32.
- Davey, K.G. (1958), The migration of spermatozoa in the female of Rhodnius prolixus Stal. J. Exp. Biol., 35, 694-701.
- Deecaraman, M. and Subramoniam, T. (1980), Male reproductive tract and accessory glands of a stomatopod, Squilla holoschista. Int. J. Invert. Reprod., 2, 175-188.

- Deecaraman, M. and Subramoniam, T. (1983), Mating and its effect on female reproductive physiology with special reference to the fate of male accessory sex gland secretion in the stomatopod Squilla holóschista. Mar. Biol. (Berl.), 77, 161-170.
- De Loof, A. (1984), Evolution and hormones controlling reproduction. In "Advances in Invertebrate Reproduction" Vol. 3. (W. Engels, ed.) Elsevier Science Publishers, Amsterdam, pp. 139-149.
- Demusy, N. (1953), Effects de l'ablation des pedoncles oculaires sur le developpement de l'appareil genital male de Carcinus maenas Pennat. C.R. Acad. Sci. Paris., 236, 974-975.
- Drach, P. (1939), Mue et cycle d'intermue chez les Crustacés Decapodés. Ann. Inst. Oceanogr., Monaco 19, 103-391.
- Dubois, M., Gilles, K.A., Hamilton, J.K., Roberts, P.A. and Smith, F. (1956), Colorimetric method for determination of sugars and related substances. Analyt. Chem., 28, 350-356.
- Dudenhause, E.E. and Talbot, P. (1983), An ultra-structural comparison of soft and hardened spermatophores from the crayfish Pacifastacus leniusculus. Can. J. Zool., 61, 182-194.
- Efford, I.E. (1972), The distribution of the sand crabs, Hippa strigillata (Stimpson) and Hippa pacifica (Dana) in the Eastern Pacific Ocean (Decapoda, Anomura). Crustaceana, XXIII, 119-122.
- Enright, J.T. (1972), A virtuoso isopod, circa-lunar rhythms and their fine structure. J. Comp. Physiol., 77, 141-162.

- Errard, C. (1978), Influence of male accessory glands on the activity of male Calliphora vomitoria (Dipt., Calliphoridae). C.R. Hebd. Seances Acad. Sci. Paris Ser. O., 287, 639-642.
- Farfante, I.F. (1975), Spermatophores and thelyca of the American white shrimps, genus Penaeus, sub-genus Litopenaeus. U.S. Natl. Mar. Fish. Serv. Fish. Bull., 73, 463-486.
- Farmer, A.S. (1974), Reproduction in Nephrops norvegicus (Decapoda, Nephropidae). J. Zool., London, 174, 161-183.
- Farooqui, U.M. and Nagabhushanam, R. (1983), Biochemical variations during testis maturation in the marine crab, Scylla serrata (Crustacea, Decapoda, Portunidae). Rev. Bras. Biol., 43, 257-260.
- Feigl, F. and Anger, V. (1966), "Spot tests in Organic Analysis". Elsevier Publishing Company, Amsterdam, London and New York.
- Ferezou, J.P., Barbier, M. et Berreur-Bonnenfant, J. (1978), Biosynthèse de la farnesylacetone (E.E.) par les glandes androgènes du crabe Carcinus maenas. Helvetica Chimica Acta, 61, 669-674.
- Ferezou, J.P., Berreur-Bonnenfant, J., Tekitek, A., Rojas, M., Barbier, M., Suchy, M., Wipf, H.K. and Meusy, J.J. (1977), Biologically active lipids from the androgenic gland of the crab, Carcinus maenas. In "Marine Natural Products Chemistry" (D.J. Faulkner and W.H. Fenical eds.) Plenum Publishing Corporation, New York, 1, 361-365.

- Feulgen, R. and Rossenbeck, H. (1924), Mikroskopisch-Chemischer Nachweis einer Nucleinsäure vom Typus des Thymonucleinsäure und die darauf beruhende elektive Färbung von Zellkernen in mikroskopischen preparaten. Z. Phys. Chem., 135, 203.
- Fielder, D.R. (1964), Spiny lobster, Jasus lalandii (H. Milne Edwards) in South Australia, II. Reproduction. Aust. J. Mar. Freshw. Res., 15, 133-144.
- Fischer, H.O.L. (1945), Harvey Lect., 40, 156, as cited in Mann (1964).
- Fodor, G., Arnold, R., Mohacsi, T., Karle, I. and Flippen-Anderson, J. (1983), A new role for L-ascorbic acid: Michael donor to α, β -unsaturated carbonyl compounds. Tetrahedron, 39, 2137-2145.
- Foulks, N.B. and Hoffman, D.L. (1974), The effect of eyestalk ablation and β -ecdysone on RNA synthesis in the androgenic glands of the protandric shrimp, Pandalus platyceros Brandt. Gen. Comp. Endocrinol., 22, 439-447.
- Fraenkel-Conrat, H., Bean, R.S. and Lineweaver, H. (1949), Essential groups for the interaction of ovomucoid and trypsin and for tryptic activity. J. Biol. Chem., 177, 385-403.
- Friedel, T. and Gillott, C. (1977), Contributions of male produced proteins to vitellogenesis in Melanoplus sanguinipes. J. Insect Physiol., 23, 145-151.
- Fryer, G. (1960), The spermatophores of Dolops ranarum Crustacea, Branchiura: their structure, formation and transfer. Quart. J. Micr. Sci., 101, 407-432.

- Gagosian, R.B., Bourbonniere, R.A., Smith, W.B., Couch, E.F., Blanton, C. and Novak, W. (1974), Lobster moulting hormones. Isolation and biosynthesis. Experientia, 30, 723-724.
- Gangotri, M.S., Vasantha, N. and Venkatachari, S.A.T. (1971), Sexual maturity and breeding behaviour in the freshwater crab, Barytelphusa guerini Milne Edwards. Proc. Indian Acad. Sci., 87, (Animal sciences -3) No.9, 195-201.
- Gavella, M. (1983), A simple automated method for determination of citric acid levels in semen. Int. J. Androl., 6, 585-591.
- Gilgan, M.W. and Idler, D.R. (1967), Gen. Comp. Endocrinol., 9, 319, as cited in "Chemical Zoology" Vol. V. (M. Florkin and B.T. Scheer, eds.), Academic Press, New York, (1970).
- Gillott, C. (1985), Arthropoda- Insecta: In "Reproductive Biology of Invertebrates" Vol. III. Accessory sex glands (K.G. Adiyodi and R.G. Adiyodi, eds.) John Wiley and Sons, U.K. (in press).
- Gillott, C. and Friedel, T. (1977), Fecundity enhancing and receptivity inhibiting substances produced by male insects: A review. In "Advances in Invertebrate Reproduction" Vol. I (K.G. Adiyodi and R.G. Adiyodi, eds.) Perlam-Kenoth, Karivellur, India, pp. 199-218.
- Gillott, C., Venkatesh, K. and Couche, G.A. (1984), Juvenile hormone control of protein accumulation in accessory gland components of male Melanoplus sanguinipes (Insecta: Orthoptera). In "Advances in Invertebrate Reproduction", Vol. 3, (W. Engels, ed.) Elsevier Science Publishers, Amsterdam, pp. 582.

- Gohr, M. and Seuty-Grosset, C. (1984), Circadian rhythms in the Crustacean Isopod Porcellio dilatatus vitellogenesis. In "Advances in Invertebrate Reproduction" Vol. 3. (W. Engels, ed.), Elsevier Science Publishers, Amsterdam, pp. 584.
- Gomez, R. (1965), Acceleration of development of gonads by implantation of brain in the crab Paratelphusa hydrodromous. Naturwissenschaften, 9, 216.
- Goudeau, M. (1982), Fertilization in a crab (Carcinus maenas) 1. Early events in the ovary, and cytological aspects of the acrosome reaction and gamete contacts. Tissue Cell, 14, 97-112.
- Greenspan, B.N. (1982), Semi-monthly reproductive cycle in male and female fiddler crabs, Uca pugnax. Anim. Behav., 30, 1084-1092.
- Greenwood, J.G. (1972), The male reproductive system and spermatophore formation in Pagurus novae-zealandiae (Dana) (Anomura, Paguridae). J. Nat. Hist., London, 6, 561-574.
- Grodner, M.L. (1979), Fine structure of the spermathecal gland of the cotton boll weevil, Anthonomus grandis (Boheman) (Coleoptera, Curculionidae). Int. J. Insect Morphol. Embryol., 8, 51-58.
- Guary, J.B. and Kanazawa, A. (1973), Distribution and fate of exogenous cholesterol during the moulting cycle of the prawn, Penaeus japonicus Bate. Comp. Biochem. Physiol., 46, 5-10.
- Gundevia, H.S. and Ramamurty, P.S. (1977), The male accessory reproductive glands and spermatophore in Hydrophilus olivaceus (Polyphaga - Coleoptera). Z. Mikrosk. Anat. Forsch., 91, 475-492.

- Haley, S.R. (1972), Reproductive cycling in the ghost crab, Ocypode quadrata (Fabr.) (Brachyura, Ocypodidae). Crustaceana, XXIII, 1-11.
- Haley, S.R. (1984), Spermatogenesis and spermatophore production in the Hawaiian red lobster, Enoplometopus occidentalis (Crustacea, Nephropidae). J. Morphol., 180, 181-194.
- Hallez, P. (1874), Compt. Rend. Acad. Sci., 70, 243, as cited in Brown (1966).
- Happ, G.M. (1984), Structure and development of male accessory glands in insects. In "Insect Ultrastructure" Vol. 2. (R.C. King and H. Akai, eds.), Plenum Press, New York, pp. 365-396.
- Hartmann, R. (1971), Endocrine factors influencing the male accessory glands and ovaries of the grasshopper Gomphocerus rufus L. (Orthoptera, Acrididae). Z. Vergl. Physiologie., 74, 190-216.
- Hasegawa, Y. and Katakura, Y. (1983), Masculinization of WW females in the isopod crustacean, Armadillidium vulgare. Annot. Zool. Jpn., 56, 163-166.
- Hebbar, C.K. (1982), "Studies on the Accessory Sex Glands and their Secretions in Some Mandibulate Arthropods!" M. Phil. Dissertation, Calicut University, Kerala, India.
- Herman, W.S. (1975), Endocrine regulation of posteclosion enlargements of the male and female reproductive glands in monarch butterflies. Gen. Comp. Endocrinol., 26, 534-540.
- Herrick, F.H. (1909), Natural history of the American lobster. Bull. U.S. Bur. Fish., 29, 149-408.

- Hinsch, G.W. (1971), Penetration of the oocyte by spermatozoa in the spider crab. J. Ultrastructure Res., 35, 86-97.
- Hinsch, G.W. and Walker, M.H. (1974), The vas deferens of the spider crab, Libinia emarginata. J. Morphol., 143, 1-20.
- Hiss, E.A. and Fuchs, M.S. (1972), The effect of matrone on oviposition in the mosquito Aedes aegypti. J. Insect Physiol., 18, 2217-2227.
- Hoffman, D.L. (1968), Seasonal eyestalk inhibition of the androgenic glands of a protandric shrimp. Nature, 218, 170-172.
- Hoffman, D.L. (1969), The development of the androgenic glands of a protandric shrimp. Biol. Bull., 137, 286-296.
- Horstmann, E. (1970), The acrosome complex of the diplode, Spirostreptus sp. In "Comparative Spermatology" (B. Baccetti, ed.), Academic Press, New York, pp. 255-262.
- Huggin, C., Scott, W.W. and Heinen, J.H. (1942), Amer. J. Physiol., 136, 467, as cited in Mann (1964).
- Iida, I. and Kawai, T. (1957), Bull. Fac. Agric. Shizuoka Univ. Japan, 7, 21, as cited in Mann (1964).
- Ilysov, I. (1933), Trans. Vet. Path. Orenburg Vet. Inst. 1, 48, cf. Anim. Breed. Abstr., 3, 479, as cited in Mann (1964).
- Iris, J.M. and Sin, F.Y.T. (1984), Relationships between weight changes, protein content and protein synthesis in the left colleterial gland during the reproductive cycle of the cockroach, Periplaneta americana. Comp. Biochem. Physiol. B. Comp. Biochem., 78, 99-108.

- Jagota, S.K., Praveen Kumar and Dani, H.M. (1982), In vivo effect of p,p'-DDT and ascorbic acid on rat liver microsomes. Res. Bull. Punjab Univ. Sci., 33, 65-68.
- James, M.O. and Shiverick, K.T. (1984), Cytochrome P-450-dependent oxidation of progesterone, testosterone and ecdysone in the spiny lobster, Panulirus argus. Arch. Biochem. Biophys., 233, 1-9.
- Joel, D.R. and Sanjeeva Raj, P.J. (1982), The breeding of 3 edible portunid crabs of Pulicat Lake. In "Progress in Invertebrate Reproduction and Aquaculture" (T. Subramoniam and Sudha Varadarajan, eds.) Indian Society of Invertebrate Reproduction, Madras, pp. 135-140.
- Johri, L.W. and Smith, J.D. (1956), A histochemical approach to the study of Helminth morphology. Parasitology, 46, 107-116.
- Johnston, M.A. and Davies, P.S. (1972), Carbohydrates of the hepatopancreas and blood tissues of Carcinus. Comp. Biochem. Physiol., 41, 433-443.
- Jones, D.A. (1972), Aspects of the ecology and behaviour of Ocypode ceratophthalmus (Pallas) and O. kuhlii de Haan (Crustacea: Ocypodidae) J. Exp. Mar. Biol. Ecol., 8, 31-43.
- Joshi, P.C. and Khanna, S.S. (1982), Structure and seasonal changes in the testes of a freshwater crab, Potamon koolooense. Proc. Indian Acad. Sci. Anim. Sci., 91, 439-450.
- Judson, P., Kishen Rao, B., Thakur, S.S. and Divakar, B.J. (1981), Male accessory reproductive glands in the bug, Dysdercus similis: Effect of a juvenile hormone analogue. I Indo-Pacific Symp. Invert. Reprod., Aurangabad, pp.24.

- Kaestner, A. (1970), "Invertebrate Zoology" Vol. III. Interscience Publishers, New York and London.
- Kalyanram, A.J. and Chandran, M.R. (1980), A note on the occurrence of a male accessory sex gland in the land crab, Cardisoma carnifex (Herbst). First All India Symp. Invert. Rep., Madras, pp. 67.
- Kalyanram, A.J. and Chandran, M.R. (1982), On the occurrence of a male accessory sex gland in the land crab, Cardisoma carnifex (Herbst). In "Progress in Invertebrate Reproduction and Aquaculture" (T. Subramoniam and Sudha Varadarajan, eds.) Indian Society of Invertebrate Reproduction, Madras, pp.61-67.
- Kanazawa, A. and Teshima, S. (1971), In vivo conversion of cholesterol to steroid hormones in the spiny lobster, Panulirus japonica. Bull. Jap. Soc. Sci. Fish., 37, 891-897.
- Kasaoka, L.D. (1974), The male genital system in two species of mysid crustacea. J. Morphol., 143, 259-284.
- Katakura, Y., Fujimaki, Y. and Unno, K. (1975), Partial purification and characterization of androgenic gland hormone from the isopod crustacean, Armadillidium vulgare. Annot. Zool. Japon., 48, 203-209.
- Katakura, Y. and Hasegawa, Y. (1983), Masculinization of females of the isopod crustacean, Armadillidium vulgare, following injections of an active extract of the androgenic gland. Gen. Comp. Endocrinol., 49, 57-62.
- Kater, S.B. and Spaziani, E. (1971), Incorporation of ^{14}C -cholesterol into crab cholesterol pools and ecdysones as a function of the moult cycle, Am. Zool., 11, 672.

- King, D.S. (1964), Fine structure of the androgenic gland of the crab Pachygrapsus crassipes. Gen. Comp. Endocrinol., 4, 533-544.
- King, J.E. (1948), A study of the reproductive organs of the common marine shrimp, Penaeus setiferus (Linnaeus). Biol. Bull., 94, 244-262.
- Krishnakumar, R. (1985), "Studies on Spermatheca of Some Decapod Crustaceans". Ph.D. Thesis, Calicut University, Kerala, India.
- Krishnakumar, R., Vijayalakshmi, V.R. and Adiyodi, K.G. (1979), Uptake of yolk proteins by developing crustacean oocytes as revealed by trypan blue technique, All India Workshop Crust. Reprod. Biol., Calicut Univ., pp. 29-30.
- Kulkarni, A.B. and Utkar, V.N. (1981), Studies on the endocrine control of albumen gland in the land snail, Cryptozona semirugata (Beck). I Indo-Pacific Symp. Invert. Reprod., Aurangabad, pp. 21.
- Kulkarni, G.K., Nagabhushanam R. and Joshi, P.K. (1984), Neuroendocrine control of reproduction in the male penaeid prawn, Parapenaeopsis hardwickii (Crustacea, Decapoda, Penaeidae). Hydrobiologia, 108, 281-290.
- Kurup, K.N.P. (1983), "Some Aspects of Limb Regeneration in the Freshwater Crab, Paratelphusa hydrodromous (Herbst)" . Ph.D. Thesis, Calicut University, Kerala, India.
- Kurup, K.N.P. and Adiyodi, R.G. (1981), The programming of somatic growth and reproduction in the crab, Paratelphusa hydrodromous (Herbst). Int. J. Invert. Reprod., 3, 27-39.
- Kurup, K.N.P. and Adiyodi, R.G. (1984), Multiple Limb autotomy can trigger either ovarian growth or somatic growth in the freshwater crab, Paratelphusa

- hydrodromous. Gen. Comp. Endocrinol., 56, 433-443.
- Labbe, A. (1904), Compt. Rend. Acad. Sci., 138, 96,
as cited in Brown (1966).
- Lange, A.B. and Loughton, B.G. (1984), An analysis of
the secretions of the male accessory reproductive
gland of the African migrating locust. Int. J.
Invert. Reprod. Dev., 7, 73-81.
- Langreth, S.G. (1969), Spermatogenesis in Cancer
crabs. J. Cell Biol., 43, 575-603.
- Leachy, M.G. (1973a), J. Ent. (a), 48, 69, In "Inverte-
brate Reproduction" (K.K. Nayar), Oxford and IBH
Publishing Co., New Delhi, (1977).
- Leachy, M.G. (1973b), J. Ent. (a), 48, 79. In "Inverte-
brate Reproduction" (K.K. Nayar) Oxford and IBH
Publishing Co., New Delhi, (1977).
- Lee, Y.P. and Takahashi, T. (1966), An improved
calorimetric determination of amino acids with the
use of ninhydrin. Analyt. Biochem., 14, 71-77.
- Legrand, J.J. and Juchault, P. (1970), Controle de la
sexualite chez les crustaces isopodes gonochoriques
et hermaphrodites. Bull. Sc. Zool. Pr., 95, 551-563.
- Leopold, R.A. (1976), The role of male accessory glands
in insect reproduction. Annu. Rev. Entomol.,
21, 199-221.
- Levinson, S.A. and MacFate, R.P. (1969), "Clinical
laboratory diagnosis," Lea and Febiger, London.
- Loher, W. (1984), Behavioral and physiological changes
in cricket - females after mating. In "Advances
in Invertebrate Reproduction" Vol. 3 (W. Engels,
ed.), Elsevier Science Publishers, Amsterdam,
pp. 189-201.

- Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951), Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193, 265-275.
- Lumsden, R.D. (1966), Cytological studies on the absorptive surface of cestodes. II. The synthesis and intracellular transport of protein in strobilar integument of Hymenolepis diminuta. Z. Parasitenkd., 28, 1-13.
- Lundquist, F. (1947), Acta, Physiol. Scand., 14, 263, as cited in Mann (1964).
- Lynn, J.W. and Clark Jr., W.H. (1983a), A morphological examination of Sperm-egg interaction in the fresh-water prawn, Macrobrachium rosenbergii. Biol. Bull. (Woods Hole), 164, 446-458.
- Lynn, J.W. and Clark Jr., W.H. (1983b), The fine structure of the sperm of the fresh-water prawn, Macrobrachium rosenbergii. Biol. Bull. (Woods Hole), 164, 459-470.
- Macleod, J. (1941), Endocrinology, 29, 583, as cited in "Acid mucopolysaccharide levels of rabbit fallopian tube, uterus and cervix: Effect of ovariectomy and estrogen treatment (A. Kushwah, D.N. Gupta, V.P. Kamboj and J.N. Karakun) Indian J. Exp. Biol., 18 (1980) 45-46
- Macleod, J. (1946), In "The Problem of Fertility" (E.T. Engle, ed)., Princeton, Princeton University Press, pp. 154.

- Macleod, J. (1951), J. Gen. Physiol., 34, 705, as cited in Mann (1964).
- Malek, S.R.A. and Bawab, F.M. (1971), Tanning in the spermatophore of a crustacean (Penaeus trisulcatus). Experientia, 27, 1098.
- Malek, S.R.A. and Bawab, F.M. (1974), The formation of the spermatophore in Penaeus kerathurus (Forsk., 1775) (Decapoda, Penaeidae). I. The initial formation of a sperm mass. Crustaceana, 26, 273-285.
- Mann, T. (1953), Ciba Found. Symp. on Mammalian Germ Cells. London Churchill, P.l, as cited in Mann (1964).
- Mann, T. (1954), Proc. Roy. Soc. B. 142, 21, as cited in Mann (1964).
- Mann, T. (1964), "The Biochemistry of Semen and of the Male Reproductive Tract". Methuen and Co. Ltd., London.
- Mann, T. (1984), Crustacea. In "Spermatophores: Development, Structure, Biochemical Attributes and Role in the Transfer to Spermatozoa". Springer-Verlag Berlin, Heidelberg, New York, Tokyo, 135-140.
- Masson, J.C. (1970), Egg-laying in the western North American crayfish, Pacifastacus trarbridgii (Stimpson) Decapode, Astacidae). Crustaceana, 19, 37-44.
- Mathad, S.G. (1983), "Biochemistry and Physiology of Semen in Freshwater Crabs", Ph.D. Thesis, Calicut University, Kerala, India.
- Mathad, S.G. and Adiyodi, K.G. (1979), On the histophysiology of the male sex duct and spermatophore formation in Paratelphusa hydrodromous. Proc. All India Workshop Crust. Reprod. Biol., Calicut Univ., pp.28-29.

- Mathad, S.G. and Adiyodi, K.G. (1980), Composition of Semen in Barytelphusa cunicularis. Proc. First All India Symp. Invert. Reprod., Madras, pp. 72.
- Mathad, S.G. and Adiyodi, K.G. (1981), Endocrine control of semen production in the crab Paratelphusa hydrodromous. Proc. I Indio-Pacific Symp. Invert. Reprod., Aurangabad, pp. 41-42.
- Matthews, D.C. (1951), Origin, development and nature of spermatophoric mass of the spiny lobster, Panulirus penicillatus (Oliver). Pacific Sci., 5, 359-371.
- Matthews, D.C. (1954a), The development of spermatophoric mass of the rock lobster, Parribacus antarcticus (Lund). Pacific Sci., 8, 28-34.
- Matthews, D.C. (1954b), A comparative study of the spermatophores of three scyllarid lobsters (Parribacus antarcticus, Scyllarides squamosus and Scyllarus martensii). Quart. J. Micr. Sci., 95, 205.
- Matthews, D.C. (1957), Further evidence of Anomuran non-pedunculate spermatophores. Pacific Sci., 11, 380-385.
- Mckenzie, F.F., Miller, J.C. and Bauguess, L.C. (1938), Res. Bull. Mo. Agric. Exp. Sta., NO.279, as cited in Mann (1964).
- Melampy, R.M., Cavazos, L.F. and Duncan, G.W. (1955), In "Michigan State University Centennial Symposium on Reproduction and Infertility". Michigan University: East Lansing, as cited in Mann (1964).
- Meusy, J.J. (1965), Contribution de la microscopie electronique a l' etude de la physiologie des glands androgenes d' Orchestia gammarella Pallas (Crustace, Amphipode) et de Carcinus maenas L. (Crustace, Decapode). Zool. Jb. Physiol. Bd., 71, 608-623.

- Mirajkar, M.S., Sarojini, R. and Nagabhushanam, R. (1983), Biochemical changes associated with the reproductive cycle in the male freshwater prawn Macrobrachium kistnensis. Biol. Bull. India, 5, 37-41.
- Moran, W.M. and Pierce, S.K. (1984), The mechanism of Crustacean salinity tolerance: cell volume regulation by potassium and glycine effluxes. Mar. Biol. (Berl.), 81, 41-46.
- Morris, G.P. and Finnegan, C.V. (1968), Studies of the differentiating plerocercoid cuticle of Schistocephalus solidus, I. Histochemical analysis of cuticle development. Can. J. Zool., 46, 115-121.
- Morrison, P.E., Venkatesh, K. and Thompson, B. (1982), The role of male accessory gland substance on female reproduction with some observations of spermatogenesis in the stable fly (Stomoxys calcitrans). J. Insect Physiol. 28, 607-614.
- Mothes, U. and Seitz, K.A. (1981), The transformation of male sex cells of Tetranychus urticae K. (Acari, Tetranychidae) during passage from the testis to the oocytes: an electronmicroscopic study. Int. J. Invert. Reprod., 4, 81-94.
- Mouchet, S. (1931), Spermatophores des Crustacés décapodes, Anomures et Brachyours et castration parasitaire chez quelques Pagures. Ann. St. Oceanogr. Salambo., 6, 1-203.
- Muthuraman, Al. (1985), "Biochemistry and Physiology of Semen in Penaeid Prawns". Ph.D. Thesis in preparation, Calicut University, Kerala, India.

- Muthuraman, Al. and Adiyodi, K.G. (1980), Histophysiology of the reproductive system of the male prawn Penaeus indicus (H.M. Edwards). Proc. First All India Symp. Invert. Reprod., Madras, pp.48.
- Muthuraman, Al. and Adiyodi, K.G. (1981), Seasonal changes in the composition of semen in the prawn Penaeus indicus. Proc. First Indo-Pacific Symp. Invert. Reprod., Aurangabad, pp.59-60.
- Muthuraman, Al. and Adiyodi, K.G. (1983), Semen production in the Indian prawn, Penaeus indicus. Proc. First Int. Bienn. Conf. Warm. Water Aquacult., Hawaii, U.S.A., pp.12.
- Muthuraman, Al. and Adiyodi, K.G. (1984), Some observations on the composition of semen in the Indian Prawn, Penaeus indicus. Inter. Conf. Biol. Benthic Marine Organisms, Aurangabad, pp. 10.
- Nagabhushanam, R. and Kulkarni, G.K. (1982), Reproductive biology of the female of a penaeid prawn, Parapenaeopsis hardwickii. Indian J. Fish., 29, 151-15
- Naylor, E. (1972), "British Marine Isopods - Keys and Notes for the Identification of the Species." Academic Press, New York and London.
- Nelson, K., Hedgecock, D. and Borgeson, W. (1983), Photoperiodic and ecdysial control of vitellogenesis in lobsters (Homarus) (Decapoda, Nephropidae). Can. J. Fish. Aquat. Sci., 40, 940-947.
- Newstead, J.D. and Dronfield, E.Z. (1965), Epithelial structure in the anterior segment of the vas deferens on an isopod Porcellio scaber (Latrielle). Z. Zellforsch., 68, 795-817.

- Novak, A.F., Blum, M.S., Taber, S. and Liuzzo, J.A. (1960), Ann. Ent. Soc. Amer., 53, 841, as cited in Mann (1964).
- Odhiambo, T.R. (1971), Tissue Cell, 2, 309, as cited in K.G. Adiyodi and R.G. Adiyodi (1975).
- Odhiambo, T.R., Kokwaro, E.D. and Sequeira, L.M. (1983), Histochemical and ultrastructural studies of the male accessory reproductive glands and spermatophore of the tsetse, Glossina morsitans morsitans. Insect Sci. Appl., 4, 227-236.
- Ono, Y. (1965), On the ecological distribution of ocypodid crabs in the estuary. Mem. Fac. Sci. Kyushu University, Series E (Biology), 4, 1-60.
- Oser, B.L. (ed.) (1965), "Hawk's Physiological Chemistry". McGraw-Hill, New York.
- Ōtsū, T. (1963), Bihormonal control of sexual cycle in the fresh water crab Potamon dehaani. Embryologica, 8, 1-20.
- Pandey, R.K. and Pandey, S.K. (1985), Seasonal fluctuations in lipid and cholesterol content of ovary and testes in relation to annual sexual cycle in Mystus M. vittatus under the stress of the Agro-chemical NPK. Proc. 72nd Ind. Sc. Cong. Part-III: Abstracts. pp.125.
- paul, A.J. (1984), Mating frequency and viability of stored sperm in the Tanner crab, Chionoecetes bairdi (Decapoda, Majidae). J. Crustacean Biol., 4, 375-381.
- paulraj, S. (1980), "Relative Growth, Tolerance to Desiccation and Autotomy of Two Species of Ocypode Crabs Showing Different Degrees of Terrestrialness". Ph.D. Thesis, University of Madras, Madras, India.

- Payen, G.G. (1972), Etude ultrastructurale de la dégénérescence cellulaire dans la glande androgène du crabe Ocypode quadrata (Fabricius). Z. Zellforsch., 129, 370-385.
- Payen, G.G., Chim, L., Laubier-Bonichon and Charniaux-Cotton, H. (1981), The androgenic gland of the shrimp Penaeus japonicus Bate, description, role and control by the eyestalks. Gen. Comp. Endocrinol., 46, 384.
- Payen, G.G., Costlow, J.D. and Charniaux-Cotton, H. (1971), Etude comparative de l' ultrastructure des glands androgenes de crabes normaux et pedonculectomises pendant la vie larvaire ou apres la puberte chez les especes: Rhithropanopsus harisii (Gould) et Callinectes sapidus Rathbun. Gen. Comp. Endocrinol., 17, 526-542.
- Pearse, A.G.E. (1968), "Histochemistry: Theoretical and Applied" . Vol. I. J. and A. Churchill, London.
- Pearson, P.J. and Walker, M.H. (1975), Alteration of cytochrome c oxidase activity during spermatogenesis in Carcinus maenas. Cell Tissue Res., 164, 401-410.
- Peferoen, M. and De Loof, A. (1983), Proteins secreted by the accessory reproductive glands of the Colorado potato beetle, Leptinotarsa decemlineata. Ann. Soc. R. Zool. Belg., 113 (Suppl.) 291-300.
- Peferoen, M. and De Loof, A. (1984a), Origin of the proteins secreted by the accessory reproductive glands of the Colorado potato beetle, Leptinotarsa decemlineata. In "Advances in Invertebrate Reproduction-3" (W. Engels ed.) Elsevier Science Publishers, Amsterdam, New York, Oxford, pp. 623.

- Peferoen, M. and De Loof, A. (1984b), Intraglandular and extraglandular synthesis of proteins secreted by the accessory reproductive glands of the Colorado potato beetle, Leptinotarsa decemlineata. Insect Biochem., 14, 407-416.
- Perrin-Waldemer, C. and Stepien, G. (1984), The in vitro kinetics of protein and nucleic acid synthesis in sex accessory glands of mated and unmated males of Drosophila melanogaster. In "Advances in Invertebrate Reproduction-3." (W. Engels ed.) Elsevier Science Publishers Amsterdam, New York and Oxford, pp. 624.
- Perrin-Waldemer, C. and Stepien, G. (1985), The in vitro synthesis kinetics of proteins and RNA in accessory sex glands of mated and unmated males of Drosophila melanogaster. Int. J. Invert. Reprod. Dev., 8, 93-101.
- Phillips, P.H., Lardy, H.A., Heiser, E.E. and Ruppel, I.W. (1940), J. Dairy Sci., 23, 873, as cited in Mann (1964).
- Pickford, R., Ewen, A. and Gillott, C. (1969), Can. J. Zool., 47, 1199, In "Invertebrate Reproduction" (K.K. Nayar) Oxford and IBH Publishing Co., New Delhi, Bombay, Calcutta, 1977.
- Piironen, J. and Hyvarinen, H. (1983), Composition of the milt of some teleost fishes. J. Fish Biol., 22, 351-362.
- Pillai, C.R.M. (1982), "Some Aspects of Reproductive Biology of the Marine crab, Ocypode platytarsis". M. Phil. Dissertation, Calicut University, Kerala, India.
- Pillay, K.K. and Nair, N.B. (1968), Observation on the reproductive cycles of some crabs from the South-West coast of India. J. Mar. Biol. Ass., India, 10, 2.
- Pillay, K.K. and Nair, N.B. (1971), The reproductive cycle of three decapod crustaceans from the South-West coast of India. Curr. Sci., 40, 161-162.

Pilyashenko-Novokhatnyi, A.I., Monosov, E.Z., Grigoryan, A.N. and Gvozdev, R.I. (1983), The investigations of methane monooxygenase from Methylococcus capsulatus. Izv. Akad. Nauk. SSSR. Ser. Biol., Q (4), 589-598.

Pochon-Masson, J. (1968a), L' ultrastructure des spermatozoids vesiculaires chez les crustaces decapodes avant et au cours de leur devagination experimentale I. Brachyours et anomours. Annls. Sci. Nat., 10, 1-100.

Pochon-Masson, J. (1968b), L' ultrastructure des spermatozoids vesiculaires chez les crustaces decapodes avant et au cours de leur devagination experimentale II. Macrours. Annls. Sci. Nat., 8, 1-188.

Pochon-Masson, J. (1983), Arthropoda-Crustacea. In "Reproductive Biology of Invertebrates" Vol. II. Spermatogenesis and Sperm Function (K.G. Adiyodi and R.G. Adiyodi, eds.), John Wiley and Sons, Chichester, pp. 407-449.

Pochon-Masson, J. and Gharagozlou-van-Ginneken, I.D. (1977), Peculiar features of the sperm of an harpacticoid copepod, Tisbe holothuriae. In "Advances in Invertebrate Reproduction" Vol. I (K.G. Adiyodi and R.G. Adiyodi, eds.) Peralam-Kenoth, Karivellur, India, pp. 61-74.

Pochon-Masson, J., Payen, G.G., Portemer, C. and Chatagner, F. (1984), Variations of taurine concentration in the ovaries and hepatopancreas of the female crab Cracinus maenas L. (Decapoda, Brachyura) during the different phases of its genital activity. Int. J. Invert. Reprod. Dev., 7, 127-133.

Poole, H.K. and Edwards, J.F. (1972), Experientia, 28, 235, as cited in Taber (1977).

- , A.E. (1974), Microbial activation of prophenoloxidase from immune insect larvae. Nature, 251, 610-613.
- ckenbush, L.S. and Hennkind, W.F. (1983), Partial characterization of eyestalk hormones controlling moult and gonadal development in the spiny lobster, Panulirus argus. J. Crust. Biol., 3, 34-44.
- l, V.G. and Crăcium, C. (1976), The ultrastructure of the androgenic gland in Porcellio scaber Latr. (Terrestrial Isopods). Cell Tissue Res., 175, 245-263.
- , V.P., Ravindranath, M.H. and Ramalingam, K. (1976), Studies on autotomy in crustaceans. II. Changes in haemolymph sugars of shore crab Ocypode platytarsis in response to autotomy and bleeding stress. Physiological Zool., 49, 389-397.
- mma, P. (1975), "Physiology of Growth and Reproduction in Oryctes rhinoceros (L)", Ph.D. Thesis, Calicut University, Kerala, India.
- murthi, R., Raghupathi, M. and Chandrasekharan, V. (1983), Observations of the breeding biology of the edible freshwater crab, Oziotelphusa senex senex (Fabricius). Proc. First Int. Biennial Conf. Warmwater Aquacult., Hawaii, pp.17.
- subramaniam, K. (1979), Qualitative and quantitative studies on proteins of reproductive glands and egg envelopes of the snail Achatina fulica. Indian J. Exp. Biol., 17, 1219-1223.

- Pye, A.E. (1974), Microbial activation of prophenoloxidase from immune insect larvae. Nature, 251, 610-613.
- Quackenbush, L.S. and Hennkind, W.F. (1983), Partial characterization of eyestalk hormones controlling moult and gonadal development in the spiny lobster, Panulirus argus. J. Crust. Biol., 3, 34-44.
- Radu, V.G. and Crăciun, C. (1976), The ultrastructure of the androgenic gland in Porcellio scaber Latr. (Terrestrial Isopods). Cell Tissue Res., 175, 245-263.
- Raja, V.P., Ravindranath, M.H. and Ramalingam, K. (1976), Studies on autotomy in crustaceans. II. Changes in haemolymph sugars of shore crab Ocypode platytarsis in response to autotomy and bleeding stress. Physiological Zool., 49, 389-397.
- Rajamma, P. (1975), "Physiology of Growth and Reproduction in Oryctes rhinoceros (L)", Ph.D. Thesis, Calicut University, Kerala, India.
- Ramamurthi, R., Raghupathi, M. and Chandrasekharan, V. (1983), Observations of the breeding biology of the edible freshwater crab, Oziotelphusa senex senex (Fabricius). Proc. First Int. Biennial Conf. Warmwater Aquacult., Hawaii, pp.17.
- Ramasubramaniam, K. (1979), Qualitative and quantitative studies on proteins of reproductive glands and egg envelopes of the snail Achatina fulica. Indian J. Exp. Biol., 17, 1219-1223.

- Ranganathan, L.S. (1977), Endocrine regulation of development and differentiation of the accessory reproductive gland of male Plebeiogryllus guttiventris. In "Advances in Invertebrate Reproduction", Vol. I (K.G. Adiyodi, and R.G. Adiyodi, eds.), Perlam-Kenoth, India, pp. 252-257.
- Ranganathan, L.S. (1982), Qualitative and quantitative studies of aminoacids in the accessory reproductive gland of Chrysocoris purpureus (Westw). In "Progress in Invertebrate Reproduction and Aquaculture" (T. Subramoniam and Sudha Varadarajan eds.), Indian Society of Invertebrate Reproduction, Madras, pp.44-47.
- Reaka, M.L. (1976), Lunar and tidal periodicity of moulting and reproduction in stomatopod crustacea: A selfish herd hypothesis. Biol. Bull., 150, 468-490.
- Reese, E.S. (1968), J. Exp. Mar. Biol. Ecol., 2, 307-318, as cited in Subramoniam (1977).
- Reger, J.F. (1970), Studies on the fine structure of spermatids and spermatozoa of the crab, Pinnixia sp. J. Mar. Morph., 132, 89-100.
- Reger, J.F. and Florendo, N.T. (1969a), Studies on motile, nontubule-containing filiform spermatozoa of the ostracod, Cypridopsis sp. I. Spermiogenesis. J. Ultrastructure Res., 28, 235-249.
- Reger, J.F. and Florendo, N.T. (1969b), Studies on motile, nontubule-containing, filliform spermatozoa of the ostracod Cypridopsis sp. II. Mature spermatozoa. J. Ultrastructure Res., 28, 250-258.
- Rice, P.R. and Armitage, K.B. (1974), The influence of photoperiod on processes associated with molting and reproduction in the crayfish Orconectes nais (Faxon). Comp. Biochem. Physiol., 47, 243-259.

- Riemann, J.G. (1973), J. Insect Physiol., 19, 213,
as cited in K.G. Adiyodi and R.G. Adiyodi (1975).
- Roth, L.M. and Dateo, G.P. (Jr.) (1965), Uric acid storage
and excretion by accessory sex glands of male
cockroaches. J. Insect Physiol., 11, 1023-1029.
- Rothschild, L. and Clealand, K.W. (1952), The physiology
of sea urchin spermatozoa. The nature and localization
of the endogenous substrate. J. Exp. Biol., 29, 66.
- Rousset, V., Raibaut, A., Manier, J.F. et Coste, F. (1978),
Reproduction et sexualite des copepodes parasites
de poissons. Z. Parasitenkd., 55, 73-89.
- Ryan, E.P. (1967), Structure and function of the
reproductive system of the crab Portunus sanguinolentus
(Herbst) (Brychura, Portunidae) 1. Male system.
Proc. Symp. Mar. Biol. Assn. India Ser. Z. Crustacea
pt., 2, 522-544.
- Sabeeda, K.C., Adiyodi, K.G. and Adiyodi, R.G. (1974),
Composition of the spermiducal secretion of the
crab, Paratelphusa hydrodromous (Herbst). J. Reprod.
Fert., 38, 244.
- Sainte-Marie, B. and Brunel, P. (1983), Differences in
life history and success between suprabenthic shelf
population of Arrhis phyllonyx (Amphipoda,
Gammaridea) in two ecosystems of Gulf of St.
Lawrence. J. Crust. Biol., 3, 45-69.
- Salmon M. and Hyatt, G.W. (1983), Communication. In "The
Biology of Crustacea" Vol. 7 (D.E. Bliss ed.)
Academic Press, New York, pp. 1-40.
- Samuel, K.A. (1984), "Reproductive Biology of the Land
Crab Gecarcinus stenlops with Special Emphasis on
the Female". M. Phil. Dissertation, Calicut University.

- Sandifer, P.A. and Lynn. J.W. (1981), Artificial insemination of caridean shrimp. In "Advances in Invertebrate Reproduction," (W.H. Clark Jr. and T.S. Adams, eds.), Elsevier, North Holland, pp. 271-288.
- Sapelkin, A.A. and Fedoseev, V.Ya. (1981), Structure of the male reproductive system of the tanner crab. Biol. Morya (Vladivost), 0(6), 37-43.
- Sarojini, R. and Jahagirdar, V.G. (1980), Changes of ascorbic acid content in testis and hepatopancreas of Clibanarius arethusa adapted to various photoperiod. Proc. First All India Symp. Invert. Reprod. Madras, pp.18.
- Scott, W.W. (1945), J. Urol., 53, 712, as cited in Mann (1964).
- Shannon, P. and Curson, B. (1983), Effects of cysteine and EDTA on in vitro survival at 37 celsius and fertility of diluted bovine semen. N.Z.J. Agric. Res., 26, 85-88.
- Sharma, D.C., Davis, P.S. and Sharma, P.K. (1982), Effect of ascorbic acid on biotransformation and modification of the toxicity of mercurials in goldfish (Carassius auratus). Experientia (Basel), 38, 565-567.
- Shepherd, J., Oliver, J.H. and Hall, J.D. (1982), A polypeptide from male accessory glands which triggers maturation of tick spermatozoa. Int. J. Invert. Reprod., 5, 129-137.
- Shergin, N.P. (1933), Trans. Vet. Path., Orenburg Vet. Inst., 1, 57; Anim. Breed. Abstr., 4, 270, as cited in Mann (1964).

- Sherief, D.S. (1983), Setting standards of male fertility:
1. Semen analyses in 1500 patients: A report.
Andrologia, 15, 687-692.
- Soderhall, K. (1982), Prophenoloxidase activating system
and melanization - recognition mechanism of
arthropods, a review. Dev. Comp. Immunol., 5, 601-611.
- Spaulding, J.F., (1942), The nature and formation of the
spermatophore and sperm plug in Carcinus maenas.
Quart. J. Micro. Sci., 83, 399-342.
- Spindler, K.D., K  ller, R. and O'Connor, J.D. (1980),
The role of ecdysteroids in the crustacean moulting
cycle. In "Progress in Ecdysone Research" (J.A.
Hoffman, ed.), Elsevier/North-Holland
Biomedical Press, Amsterdam, pp. 247-280.
- Sprince, H., Clarence, M.P., George, G.S. and Gonzales,
L.J. (1975), Protective action of ascorbic acid and
sulfur compounds against acetaldehyde toxicity:
Implications in alchoholism and smoking. Agents Actions,
5, 164-173.
- Sreekumar, S. (1985), "Reproductive Physiology of Male
Decapod Crustacean". Ph.D. Thesis under preparation,
Calicut University, Kerala, India.
- Sreekumar, S. and Adiyodi, R.G. (1981), Moults related
changes in spermatogenesis and semen production in
the shrimp, Macrobrachium idella. Proc. First
Indo-Pacific Symp. Invert. Reprod. Aurangabad, pp.59-60.
- Sreekumar, S. and Adiyodi, R.G. (1983), Role of eyestalk
principle(s) in spermatogenesis of the freshwater
shrimp, Macrobrachium idella. Proc. First Int. Binnial
Conf. Warm Water Aquacult. Hawaii, pp. 15-16.
- Sreekumar, S., Adiyodi, R.G. and Adiyodi, K.G. (1980),
Aspects of semen production in Macrobrachium idella.

- Sherief, D.S. (1983), Setting standards of male fertility:
1. Semen analyses in 1500 patients: A report.
Andrologia, 15, 687-692.
- Soderhall, K. (1982), Prophenoloxidase activating system
and melanization - recognition mechanism of
arthropods, a review. Dev. Comp. Immunol., 5, 601-611.
- Spaulding, J.F., (1942), The nature and formation of the
spermatophore and sperm plug in Carcinus maenas.
Quart. J. Micro. Sci., 83, 399-342.
- Spindler, K.D., K  ller, R. and O'Connor, J.D. (1980),
The role of ecdysteroids in the crustacean moulting
cycle. In "Progress in Ecdysone Research" (J.A.
Hoffman, ed.), Elsevier/North-Holland
Biomedical Press, Amsterdam, pp. 247-280.
- Sprince, H., Clarence, M.P., George, G.S. and Gonzales,
L.J. (1975), Protective action of ascorbic acid and
sulfur compounds against acetaldehyde toxicity:
Implications in alchoholism and smoking. Agents Actions,
5, 164-173.
- Sreekumar, S. (1985), "Reproductive Physiology of Male
Decapod Crustacean". Ph.D. Thesis under preparation,
Calicut University, Kerala, India.
- Sreekumar, S. and Adiyodi, R.G. (1981), Moults related
changes in spermatogenesis and semen production in
the shrimp, Macrobrachium idella. Proc. First
Indo-Pacific Symp. Invert. Reprod. Aurangabad, pp.59-60.
- Sreekumar, S. and Adiyodi, R.G. (1983), Role of eyestalk
principle(s) in spermatogenesis of the freshwater
shrimp, Macrobrachium idella. Proc. First Int. Binnial
Conf. Warm Water Aquacult. Hawaii, pp. 15-16.
- Sreekumar, S., Adiyodi, R.G. and Adiyodi, K.G. (1980),
Aspects of semen production in Macrobrachium idella.
IFS Provisional Report No.9, 113-117.

- Sreenarayanan, K.V. and Adiyodi, R.G. (1979), Seminal chemistry of the ghost crab, Ocypode ceratophthalmus: the possible role of ductal and accessory glands in the secretion of the seminal plasma. All India Workshop Crust. Reprod. Biol., 30-31.
- Sreenivasulu Chetty, P., Kalarani, V., Chandrasekhara Reddy, D. and Padmanabha Naidu, B. (1984), Circadian variations in the levels of calcium and magnesium of freshwater crab, Oziotelphusa senex senex. Geobios (Jodhpur), 11, 25-27.
- Sriramulu, V. (1982), Strategic role of accessory sex organs in pulmonate reproduction. In "Progress in Invertebrate Reproduction and Aquaculture" (T. Subramoniam and Sudha Varadarajan, eds.), Indian Society of Invertebrate Reproduction, Madras, pp. 31-43.
- Sriramulu, V. and Magdalene (1981), Influence of the optic tentacular principle in the functional differentiation of the dart sac in Cryptozona belangeri (Deshayes) (Mollusca: Gastropoda). I Indo-Pacific Symp. Invert. Reprod., Aurangabad, pp. 41-42.
- Steedman, H.F. (1950), Alcian blue 8G: A new stain for mucin. Quart. J. Micr. Sci., 91, 477-479.
- Steeves, H.R. (1963), J. Exptl. Zool., 154, 21-38, as cited in "Chemical Zoology" Vol. V (M. Florkin and B.T. Scheer, eds.), Academic Press, New York (1970).
- Strus, J. and Blejec, A. (1983), Reproductive activity in Asellus aquaticus (Crustacea, Isopoda) from Ljubljansko barje (Yugoslavia). Biol. Vestn. 31, 83-92.
- Subramoniam, T. (1977), Continuous breeding in tropical Anomuran crab, Emerita asiatica Milne Edwards from Madras coast. In "Advances in Invertebrate Reproduction" (K.G. Adiyodi and R.G. Adiyodi, eds.) Peralam-Keneth, pp. 166-174.

- Subramoniam, T. (1981), sperm transfer strategy in two intertidal Anomuran crab, Emerita asiatica and Albunea symnisia. In Proc. U.K. Meeting Int. Soc. Invert. Reprod., pp.59.
- Sukumaran, M. and Adiyodi, K.G. (1980), Studies on semen production in Ocypode platytarsis. First All India Sym. Invert. Reprod., Madras, pp.60.
- Taber, III, S. (1977), Semen of Apis mellifera: fertility, chemical and physical characteristics. In "Advances in Invertebrate Reproduction" Vol. I (K.G. Adiyodi and R.G. Adiyodi, eds.) Peralam-Kenoth, Karivellur, India, 219-251.
- Talbot, P. and Chanmanon, P. (1980), Morphological features of the acrosome reaction of lobster (Homarus) sperm and role of the reaction in generating forward sperm movement. J. Ultrastructure Res., 70, 287-297.
- Tasi, D.E., Hoa-Chia, C. and Chu-Fa, T. (1984), Total lipid and cholesterol content in the blue crab, Callinectes sapidus. Comp. Biochem. Physiol. B. Comp. Biochem., 78, 27-32.
- Tcholakian, R.K. and Reichard, S.M. (1964), A possible androgenic gland in Callinectes sapidus Rathbun. Amer. Zoologist (Abstract), 4, 383.
- Thampy, D.M. and John, P.A. (1970), On the androgenic gland of the ghost crab, Ocypode platytarsis. M. Edwards, (Crustacea: Brachyura). Acta Zoologica, 51, 203-210.
- Thampy, D.M. and John, P.A. (1973), Observations on variations in the male sex characters and their relation to the androgenic gland in the shrimp Macrobrachium idae (Heller). Acta. Zool., Stockholm, 54, 193-200.

- Tighe-Ford, D.S. (1967), Possible mechanisms for the endocrine control of breeding in a cirripede. Nature, 216, 920-921.
- Tosic, J. and Walton, A. (1945), Nature, Lond., 156, 507, as cited in Mann (1964).
- Tosic J. and Walton, A. (1946), Nature, Lond., 158, 485, as cited in Mann (1964).
- Tosic J. and Walton, A. (1950), Biochem. J., 47, 199, as cited in Mann (1964).
- Touir, A. (1977a), Physiologie des invertébrés - Données nouvelles concernat l' endocrinologie sexuelles des Crustacés Décapodes Natantia hermaphrodites et gonochoriques. II. Maintien des gonies et evolution des gametogenesis in vivo et in vitro. C.R. Acad. Sc. Paris, 284, 2515-2518.
- Touir, A. (1977b), Physiologie des invertébrés Données nouvelles concernant l'endocrinologie sexuelle des Crustacés Décapodes Natantia hermaphrodites et gonochoriques. III. Mise en évidence d'un contrôle neurohormonal du maintien de l'appareil genital mâle et des glandes androgenes excrcepar le protocerebron median. C.R. Acad. Sc. Paris, 285, 539-542.
- Tyler, A. and Rothschild, L. (1951), Proc. Soc. Exp. Biol. N.Y., 76, 52, as cited in Mann (1964).
- Uma, K. and Subramoniam, T. (1979), Histochemical characteristics of spermatophore layers of Scylla serrata (Forsk.) (Decapoda: Portunidae). Int. J. Invert. Reprod., 1, 31-40.

- Uma, K. and Subramoniam, T. (1981a), Ascorbic acid content and phosphatase activity in the seminal secretion and male reproductive system of an edible crab, Scylla serrata. First Indo-Pacific Symp. Invert. Reprod., Aurangabad, pp.63.
- Uma K. and Subramoniam, T. (1981b), An electrophoretic investigation on the protein pattern of seminal secretion in the edible crab, Scylla serrata. First Indo-Pacific Symp. Invert. Reprod., Aurangabad, pp.65.
- Varghese, A.S. (1984), "On Semen Production in the Crab, Gecarcinus stenops". M.Phil. Dissertation, University of Calicut, Kerala, India.
- Vaughn, J.C. and Thomson, L.A. (1972), A kinetic study of DNA and basic protein metabolism during spermatogenesis in the sand crab, Emerita analoga, J. Cell Biol., 52, 322-337.
- Veera Raghavan, V., Ruknudin, A. and Shahul Hameed, M.M. (1981), Electrophoretic and immunological studies of the male accessory reproductive gland proteins of Poecilocera picta Fabr. (Acrididae: Orthoptera) Int. J. Invert. Reprod., 3, 275-282.
- Veera Raghavan, V., Ruknudin, A. and Shahul Hameed, M. . (1982), Histological, histochemical and electrophoretic studies on the male accessory reproductive glands of Poecilocera picta Fabr. (Acrididae: Orthoptera). In "Progress in Invertebrate Reproduction and Aquaculture" (T. Subramoniam and Sudha Varadarajan, eds.), Indian Society of Invertebrate Reproduction, Madras, pp. 48-60.

- Vierula, M. and Rajaniemi, H. (1983), Effect of seminal plasma and calcium on the stability of the surface protein composition of ejaculated bull spermatozoa. Andrologia, 15, 436-445.
- Vogel, A.I. (1959), "A Text Book of Practical Organic Analysis". Spottis Woods, Ballantyne and Co. Ltd., London.
- Walley, L.J., White, F. and Brander, K.M. (1971), Sperm activation and fertilization in Balanus balanoides. J. Mar. Biol. Assn., U.K., 51, 489-494.
- Watras, C.J. (1983), Reproductive cycles in diaptomid copepods: Effects of temperature, photocycle and species on reproductive potential. Can. J. Fish Aquat. Sci., 40, 1607-1613.
- West, E.S., Todd, W.R., Mason, H.S. and Van Bruggen, J.T. (1966), "Text Book of Biochemistry", The Macmillan Company, London.
- Wiens, A.W. and Gilbert, L.I. (1967), Regulation of carbohydrate mobilization and utilization in Leucophaea maderae, J. Insect. Physiol., 13, 779-794.
- Wolfe, A.F. (1974), A histological and histochemical study of the male reproductive system of Artemia (Crustacea, Branchiopoda). J. Morphol., 135, 51-70.
- Wright, H.D. (1968) (M.S.) Visual displays in brachyuran crabs, field and laboratory studies, Amer. Zool., 8, 655-665, as cited in Bliss et al. (1978).

- Yasuma, A. and Ichikawa, T. (1953), Ninhydrin-Schiff and Alloxin-Schiff staining, a new histochemical method for protein. J. Lab. Clin. Med., 41, 296-299.
- Young, J.E. (1974), Variation in the timing of spermatogenesis in Uca pugnax (Smith) and possible effectors (Decapoda, Brachyura, Ocypodidae). Crustaceana, 27, 68-72.

595-580-2 SUR/c

List of research papers presented by the author in national and international symposia.

1. M. Sukumaran and K.G. Adiyodi (1980), Studies on the semen production in the Ocypode platytarsis. Proc. First All India Symp. Invert. Reprod., Madras, p.60-61.
2. M. Sukumaran and K.G. Adiyodi (1981), Semen in the ghost crab, Ocypode platytarsis: Composition and the role of the eyestalk principles in its production. Proc. U.K. Meeting Int. Soc. Invert. Reprod. p.60.
3. M. Sukumaran and K.G. Adiyodi (1984), Eyestalk influence on the histophysiology of the male tract and composition of semen in Ocypode platytarsis. In "Advances in Invertebrate Reproduction - Vol. -3," (W. Engels ed.), Elsevier Science Publishers - Amsterdam-New York-Oxford, 642.

R

NB 413

