EFFECT OF EYESTALK ABLATION ON ANDROGENIC GLAND AND MALE SECONDARY SEXUAL CHARACTERS IN *MACROBRACHIUM IDELLA* (Hilgendorf)

BY

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THESIS

Submitted in partial fulfilment of the requirement for the degree

MASTER OF FISHERIES SCIENCE

FACULTY OF FISHERIES

KERALA AGRICULTURAL UNIVERSITY

DEPARTMENT OF AQUACULTURE

COLLEGE OF FISHERIES

PANANGAD , COCHIN

1998

Dedicated
To
My Husband

DECLARATION

I hereby declare that this thesis entitled " EFFECT OF EYESTALK ABLATION ON ANDROGENIC GLAND AND MALE SECONDARY SEXUAL CHARACTERS IN *MACROBRACHIUM IDELLA* (Hilgendorf)" is a bonafide record of research work done by me during the course of research and that the thesis has not formed the basis for the award to me of any degree, diploma, associateship, or other similar title , of any other University or Society.

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ACKNOWLEDGMENTS

I would like to place on record my sincere thanks to all those who have helped me in one way or other in the successful conduct of my research programme and drafting of this thesis.

I am deeply indebted to **Dr. D.M.Thampy**, Professor and Head, Department of Aquaculture, College of Fisheries, Panangad, for his valuable guidance and constant encouragement which were the motive forces behind the timely completion of my research work. I remain grateful to him for the helpful advice and constructive suggestion given during the course of the experiment and preparation of the thesis. His keen attention and advice helped me a lot in preparation of the thesis and submitting it in the present form.

It is with great sense of gratitude I remember the name of **Dr. K.V.Jayachandran** Associate Professor, Department of Fishery Biology, College of Fisheries Panangad, for I was fortunate to have benefited from the valuable counselling, inspiration and help from him in carrying out the research smoothly and in presentation of this written document.

My thanks are due to **Dr.C.Mohanakumaran Nair**, Associate Professor, Department of Aquaculture,College of Fisheries, Panangad for being a constant source of support in my endeavours and also for his cordial and timely help during the course of the experiment .

I am indebted a great deal to **Sri.T.M.Sankaran**, Associate Professor and Head, Department of Management Studies, College of Fisheries, Panangad, not only for the help in the design of experiments and analysis of the result , but also for his scholarly and critical comments both during the experiment and preparation of thesis.

I sincerely thank **Mrs. Alphi Korath**, Assisstant Professor, Department of Management Studies, College of Fisheries, Panangad, who helped me a lot in the statistical analysis and the preparation of this thesis.

I sincerely thank **Dr.C. J.Cherian** , Associate Professor , Department of Fishery Hydrography, College of Fisheries, Panangad and **Dr. M.V.Mohan**, Assisstant Professor, Department of Aquaculture, College of Fisheries , Panangad, who helped me in drawing the diagrams.

I sincerely thank all the staff of Department of Aquaculture, College of Fisheries , Panangad,who helped me in completing the research work and offered their constructive criticism.

My friends empowered me with scientifically worth ideas, keen observation and timly help which is thankfully acknowledged.

I express my sincere thanks to the Librarians of College of Fisheries , Panangad,for their valuable co-operation and help.

I am indebted a great deal to **Sri.Vinod, Sri.Morris** and **Sri Abdul Raoof** who helped me a lot to bring this thesis in the present form.

Once again I thankfully acknowledge all persons whose wisdom and experience have been utilized in one way or another for the successful completion of this thesis.

Sherine Sonia Cubelio

CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

The freshwater prawns of the genus *Macrobrachium* have gained importance in the recent years as a candidate species for culture in the fresh water bodies as well as in the low saline areas all over the world . The rapidly growing prawn aquaculture industry , among other areas look towards crustacean endocrinology, to assist in developing techniques to increase crop yield by stimulating growth and controlled reproduction under captive conditions . Controlled breeding in captivity provides the advantage of obtaining pure seed and opens up avenues of large scale husbandry. With increasing knowledge of endocrine activity and control of gonadal development in crustaceans , the technique of eyestalk ablation is receiving greater attention as a method of inducing precocious maturation in captivity .

The mechanism of sex determination has not been studied extensively in crustaceans (Katakura , 1989) and we have only limited cytogenetical data with regard to decapods (Legrand *et al* ., 1987) . Polymorphism among a particular sex has been reported among crustaceans especially among the fresh water prawns . Thus Henderson and Mathai (1910) noticed dimorphism in the second chelipeds of males in three species of *Palaemon* , namely *P . malcolmsonii* , *P.dubius* and *P.scabriculum* . But they were not able to establish a clear correlation of the dimorphism with breeding season or as to whether the same individual remains in the same condition throughout or there is a change from one condition to another .

It has been suggested that the androgenic gland is the exclusive source of a hormone responsible for sex differentiation in crustaceans (Charniaux - cotton , 1954) . In male crustaceans - unlike vertebrates - the endocrine and gametogenic functions are clearly separated into distinct organs, the androgenic gland and the testis , respectively (Vogel and Chaniaux - cotton , 1982 ; Chamiaux - cotton and Payen , 1988) . Thus the androgenic gland serves as a unique biological model for the study of the endocrine regulation of sex - differentiation .

The androgenic gland plays an important role in crustacean sex determination as well as in the regulation of primary and secondary sexual characteristics . The importance of this gland for aquaculture research lies in the fact that , in some crustaceans , males and females differ in their growth patterns , and males are larger and grow faster than females, on the basis of the influence of male hormone produced by the androgenic gland . In *Macrobrachium rosenbergii* , the male growth

rate is considerably higher than that of the female (Sagi et al ., 1986) . However , male growth rates vary greatly (Fujimura and Okamoto ,1972 ; Smith et al ., 1978 ; Brody et al .,1980 , Malecha et al ., 1984) due to the existence of different male morphotypes within the prawn population (Ra'anan and Cohen , 1985 ; kuris et al ., 1987 ; Sagi and Ra'anan , 1988 ; Sagi et al ., 1988) . In *Macrobrachium idae* ,even among the males of same size , there is variation among cheliped length , size and spinuosity of appendix masculina and development of testis as indicated by the width of vas deferens This variation has been found to be linked with the extent of development of androgenic gland (Thampy and John , 1973) .

Most of the studies relating to eyestalk ablation have primarily been concerned with the effect of eyestalk ablation on gonadal maturation and spawning of female crustaceans . Only in a few cases , the effect of ablation on the growth and reproduction of both sexes , has been studied .

The giant freshwater prawn *M . rosenbergii* is widely cultured in Asian countries such as Thailand, Taiwan, Malaysia , Vietnam, India, Indonesia and Bangladesh and in Hawaii and S Carolina in USA . The global production of freshwater prawn increased from 7165 mt in 1986 to 16047 mt in 1995 .. However the development of freshwater prawn culture will largely depend on utilization of the other large species such as *M . malcolmsonii* , *M. acanthurus* and *M . tellinium* and many medium size species such as *M . idella* , *M . equidens* and *M . rude* . In this context species like *M .idella* , though a medium sized one , can be considered as a potential candidate (Natarajan et al ., 1979 ; Ignatius , 1989 ; Ignatius and Thampy , 1991) . *M . idella* has attractive attributes towards aquaculture in that it is tolerant to a wide range of salinity and is very hardy.

An understanding of the mechanism that controls variation in the development of secondary sex characters and its relationship with the growth of the prawn , if any , will be of great help in finding ways and means to get maximum growth and production of freshwater prawn in culture , by manipulating the stock and indulging in partial selective harvesting . The present study is intended to find out the role if any , the eyestalk endocrine has , on the control of androgenic gland , which in turn is controlling the development of primary as well as secondary sex characters and also the morphotypic variations among the males .

2 . REVIEW OF LITERATURE

2.1 Distribution of freshwater prawns

Species of the freshwater prawns of the genus *Macrobrachium* are distributed through out the tropical and subtropical regions . Of the 100 or so species known to exist today, many are cultivable and used for inland aquaculture.

In India, the genus is represented by about 40 species, out of which 15 are important from fisheries point of view. *M. rosenbergii* which is distributed from indus delta of India, China to Asian mainland is available in West Bengal , Gujarat and Kerala. *M . malcolmsonii* , the species next in importance, is restricted to India only and is found in the peninsular rivers that drain into the Bay of Bengal . Although it is available in states such as Karnataka and Maharashtra ,this species is not available in the Kerala rivers .

M. Idella , known as " slender river prawn " (Holthius , 1980) having a distribution in Indo West Pacific , East Africa , Madagascar and India , *M . Scabriculam* , distributed in Indo West Pacific, East Africa and Madagascar to India , Srilanka , Bangladesh and Sumatra and *M . equidens* distributed in Indo West Pacific , Nigeria and Madagascar to South China , New Britan and New Calidonia are the three species of medium sized *Macrobrachium* which are available in sizeable quantities in Kerala backwaters and canals. Of these , *M . idella* supports a significant fishery in many parts of Kerala , especially in Vembanad lake , where Kurup et al ., (1992) have reported an annual catch in the order of 68 .3 tonnes . Jayachandran (1984) described two subspecies of *M . idella* viz , *M . idella idella* and *M . idella georgii* . Of these *M . idella georgii* is available abundantly in rivers such as Meenachal , Manimala , Pamba and Achencoil and in stray numbers in the Pallickal river . *M . idella idella* , on the other hand , is distributed in large numbers in almost all the rivers of Kerala .

M . equidens found mostly in brackishwater areas is distributed in Kerala only north of Cochin backwater and it forms a fishery during the north - east monsoon period in the lower stretches of Korapuzha river at Elathur, Kadalundi estuary, Chettuva backwater and Azhikode (Jayachandran , 1987). Kurup et al ., (1992) reported that the availability of *M . equidens* in Vembanad lake in Kerala coincided with higher salinities .

2.2. Growth

Growth in crustaceans is under endocrine control . The x - organ sinus gland complex located in the eyestalk controls growth , reproduction , moulting , retinal pigment migration , metabolism of blood sugar , protien , lipids and fatty acids , osmo regulation , feeding and food conversion efficiency (New Comb , 1983 ; Chandry and Kalwalkar , 1984) . The moult - inhibiting hormone (M I H) , a neuropeptide originating from the x - organ and stored in the sinus gland is known to inhibit the secretion of ecdysteroids by the y - organ or moult gland .

2. 2.1 *Morphotypic differentiation and growth patterns*

2 . 2. 1 . 1. Males

Sexually mature populations of *Macrobrachium rosenbergii* are characterized by a positively skewed , bimodal weight distribution (Smith et al ., 1978 ; Karplus et al ., 1986) . The asymmetric weight distribution in males is associated with the co - existence of three distict morphotypes (Raa'nan and Cohen, 1985 ; Kuris et al ., 1987) which are characterized by physical features such as relative size, claw colour and claw length (Sandifer and Smith , 1978) as well as by behavioural characteristics such as territoriality , aggression , mobility , feeding behaviour and reproductive behaviour (Peebles , 1979) . These three morphotypes are : (1) blue clawed males (BC) also termed as bulls (Fujimura and Okamoto, 1972) which possess blue coloured spinuous claws and a high ratio of claw to body length (2) Orange clawed males (OC) possessing non - spinuous , often orange coloured claws , a low ratio of claw to body length and (3) Small males (SM) also termed runts (Fujimura and Okamoto , 1972) , smaller than the other two morphotypes , possessing delicate claws , clear or light pink colour and a low ratio of claw to body length . These morphotypes represent three phases in male development reflected by the changes of size , morphology , physiology and behaviour (Telecky , 1984 ; Raa'nan and Sagi , 1985 ; Kuris et al ., 1987 ; Sagi and Raa'nan , 1988 ; Barki et al ., 1991 a , b) . All male juveniles are reported to remain first as SM , then transform to OC male and finally into BC male in a developmental sequence when raised in isolation (Raa'nan and Cohen ,1985 ; Raa'nan and Sagi , 1985; Kuris et al ., 1987 ; Barki , 1989) . When raised in a group , however , only some males transform into the fast growing OC , while the rest remain SM whose growth is inhibited by larger individuals . According to Cohen et al . (1988) females in a mixed population may induce a larger fraction of males to enter into the more reproductively active but slower growing BC and SM types . SM males become OC males through a " weak OC intermediate " phase but OC males become BC males at a single metamorphic moult (Kuris et al ., 1987) . Raa'nan

and Cohen (1985) suggested that the first male to attain BC status would be one of the most rapidly growing OC males and it would be subsequently surpassed by other OC males in a " leap frogging " process, such that the largest BC male in the population will be the most recently metamorphosed male . They also observed that the size of BC males in a population is inversely proportional to the amount of algae adhering to their cuticles as coverage by epibionts is an indicator of time since the previous ecdysis . Karplus et al . (1991)supported the " leap frog " hypothesis and suggested that the " leap frog " growth pattern is probably due to social interactions among males , because males isolated in small cages did not follow this pattern . The " leap frog " growth pattern results in a series of differently sized BC males , whose size is positively correlated with the time of their metamorphosis . This growth pattern is achieved mainly through a delay in the transition from the fast growing OC morphotype into the slow growing BC . Thus it can be seen that the growth rate of males is highly variable (Fujimura and Okamoto , 1972 ; Smith *et al* ., 1978 ; Brody *et al* ., 1980 ; Raa'nan , 1982 ; Malecha *et al* .,1984) . The small male (SM) morphotype has a slow growth rate , while the orange claw male (OC) morphotype has a high growth rate and as OC males transform to BC morphotype, growth ceases (Raa'nan and Cohen , 1985 ; Kuris et al ., 1987 ; Raa'nan et al ., 1991) Thus the growth of male is depensatory throughout the process of morphotypic differentiation , leading to a wide range of OC and BC males.

Morphotypic differentiation has been reported in other species of *Macrobrachium* . Henderson and Mathai (1910) observed that in many , if not in all species , two forms of males are met with viz , normal males usually of considerable size ,with larger chelipeds especially developed , and the males of the second type (" male feminises ") generally smaller , but sometimes attaining the same size as normal males in which the chelipeds resemble those of females . This difference in the development of the secondary sex characters among males of *M . idae* has been reported to be linked with the extent of development of androgenic gland (Thampy and John , 1973) . Naganine and Knight (1980) noted two types of males with appendices masculinae present . These males differed in the relative length of cheliped . Jayachandran and Joseph (1988) studied the growth pattern of *M . idella* and *M . scabriculum* . They found that the males of *M . scabriculum* exhibit isometric growth , but the females of *M . scabriculum* and both sexes of *M . idella* do not exhibit isometric growth .

It may be possible that androgenic gland which controls the primary and secondary sex characters of male crustaceans has a role in the development of male morphotypes and growth variation among them , as growth rate of prawns is closely associated with morphotypic status (Raa'nan , 1982 ; Raa'nan and Cohen , 1985).

2 . 2.1 .2 Females

Growth of females of *M. rosenbergii* was found to be reduced during egg development , since a proportion of the available energy is used for the development of oocytes (Wickins and Beard , 1974) . In matured females , three different morphotypes are usually observed . Virgin female (V) , berried female (BE) and previously spawned females with open pleura . The growth rate of immature females is relatively high , approaching that of the orange claw males (Raa'nan et al ., 1991).After maturation , growth slows down considerably but does not cease . Variation in size decreases with time, because growth rate declines markedly with increasing size , whether the prawns are mature or not . Thus the model for female growth is quite different from that of male growth . Raanan et al . , (1991) reported that in the female population , parallel to the increase in average weight with time , there is a continuous decrease in the co - efficient of variation . They also found that the largest females at stocking, usually remained the largest throughout the experiment , whether they mature or not.

2 .2 .2 . ***Effect of eyestalk ablation on growth***

Eyestalk ablation as a method for accelerating growth has been documented in crabs such as *Eriochier sinensis* (koch , 1952),cray fishes such as *Astacus astacus*(Gydanco and Westin,1988). prawns such as *Macrobrachium malcolmsonii* (Murugadass et al ., 1988) , *Penaeus canaliculatus* (Choy , 1987) , *P . merguiensis* and *P . monodon* (Alikunhi et al ., 1975) . However , there are also reports where the growth rates are not significiantly affected by eyestalk ablation in the species such as *P . monodon* (Emmerson , 1983) and *Paralithodes camschatica* (Molyneaux and Sheirly , 1988) .

Koch (1952) working on the crab *Eriochier senensis* reported that the better weight increment in ablated specimens is simply the result of absorption of excess water , rejecting the possibility of real growth or tissue synthesis . Mauviout and Castell (1976) on the other hand reported real growth acceleration in ablated *Homarus* [illegible] . The difference between ablated and unablated , they could obser[illegible] , was the lower value of lipid deposition in the muscle and hepatopancreas and an accelerated growth in protien in the case of ablated specimen .Murugadass *et al.* (1988) working on the fresh water prawn *M . malcolmsonii* reported accelerated growth for destalked specimen of both sexes which they have attributed to the less energy being channelised towards exuvial production . Accelerated growth rate acheived as a result of increased weight gain was reported in the case of *Penaeus canaliculatus* (Choy , 1987) .

2 . 3 . Reproductive biology of *Macrobrachium*

2 . 3. 1. *Sexuality*

Palaemonid prawns are dioecious, the sexes are being distinguished by a number of external characters (Patwardhan , 1937) . In general, the females are smaller than males of same age. The second chelate legs of males are more elongated, stout and profusely covered with setae. The males are also characterized by the presence of appendix masculina in the endopodite of second pleopod. In females, the epimera of the abdominal segments are bigger in size and form deep recess for carrying eggs during breeding season. The male genital apertures are paired, and present on the arthrodial membrane above the coxa on the inner side of the last pair of walking legs, covered over by small tongue like flaps of integument. The female genital aperture is also paired, each being situated in a raised papilla on the inner side of the coxa of the third walking legs.

Among crustaceans dimorphism in males has been recorded in cambarine cray fishes and tanaids. Henderson and Mathai (1910) noticed dimorphism in the second chelipeds of males in three species of *Macrobrachium* namely *M . malcolmsonii* , *M. dubius* and *M . Scabriculum*. But they were not able to establish a clear correlation of the dimorphism with breeding season. Thampy and John (1973) found that a majority of the male of the fresh water prawn, *M . idae* show a hypertrophy in the length of second cheliped, the length and spinuosity of appendix masculina and the width of vas deferens, during the breeding season. This hypertrophy has only very little correlation to the size of the individual, and the above sex characters had marked variation in a population having the same body length. The hypertrophy of these structures was found to be linked with the extent of development of the androgenic gland . Thus they have suggested that there is a direct relation between the secretory activity of the androgenic gland and the hypertrophy of these structures ie, there is a " cause and effect" relation between the androgenic gland and the primary and secondary sex characters.

2.3.2 *Sex ratio*

Seasonal variation in sex ratio has been reported in the case of *Macrobrachium* spp. Raman (1967) has reported male domination in the catches especially during May - June from the Vembanad lake . Kurup et al (1992) reported a male : female ratio of 1:1.11 for *M. rosenbergii* and 1;1.29 in the case of *M .idella* from the catches from Vembanad lake . The seasonal variation was also found to be pronounced, it being 1:0.17 to 1:5.85 in the case of *M . rosenbergii*, the female dominating during September to December and males during March to June and 1: 0.78 to 1:1.69 in the case of *M . idella,* the females dominating during all months except October to December .

2.3.3 *Age and size at maturity*

Many of the palaemonids reach sexual maturity within an year . The size at first maturity of *M.rosenbergii* has been investigated by many workers. In rivers of W. Bengal it takes two years(Rajyalakshmi ,1961 and 1962) and in Kerala one year (Raman, 1967) to attain maturity.Rao(1967) recorded the mean size of 155mm as the maturity size in Hooghly estuary. Goorah and Parameswaran (1983) recorded 115 mm and 20 g (5.7 months old) . as the smallest size of berried females in ponds at Mauritius . The size at first maturity of *M . malcolmsonii* is 41mm according to Ibrahim (1962) and 40.50mm according to Sankolli and Shenoy (1980). Jayachandran (1984) reported the age of maturity of *M . idella* as 120 days. Pillay and Mohammed also reported it as 120 days under laboratory conditions .

2.3.4 *Breeding season*

The breeding season of fresh water prawns of the genus *Macrobrachium* shows considerable variation, mostly coinciding with the onset of monsoon. *M . rosenbergii* breeds from December to July in the Hoogly estuary (Rajyalakshmi, 1961,1962; Rao, 1967) and in kerala it breeds during August to December with a peak in September - November (Raman , 1967 ; Kurup *et al.* , 1992) . *M . malcolmsonii* breeds from April to November in Godaveri river system with two peaks, one in June and the other during August to October (Ibrahim, 1962). The breeding season of *M . idella* extends from August to December / January (Jayachandran , 1984) . *M . scabriculum* breeds throughout the year (Jayachandran and Joseph , 1989). Jayachandran and Joseph (1989) reported that the breeding season of *M . equidens* as from August to January / February while Pillay (1990) noted it as August to November .

2.4 Endocrine control of sex and reproduction of prawns

2.4.1 *Endocrine control of sex*

2.4.1.1 Males

In the case of male malacostracan crustaceans sex and reproduction are under the influence of androgenic gland which in turn is under the control of x-organ sinus gland system . The androgenic gland , first described by Charniaux -Cotton (1954) is usually located at the dorsomedian surface of the terminal ampoule at the distal end of the sperm duct where it forms a pyramidal cluster

of loosely arranged cordon of cells, associated with the posterior position of the ejaculatory duct (Thampy and John , 1972 , 1973 ; Sagi , 1988 ; Philip and Subramoniam , 1992).

Charniaux - Cotton (1954) was the first to suggest a regulatory role for the androgenic gland . She showed that bilateral androgenic gland ablation (andrectomy) in the amphipod *Orchestia gammarella* blocked differentiation of secondary male characteristics and resulted in decreased spermatogenesis (Charniaux - cotton , 1954) . The androgenic gland secretes a hormone which determines the development of primary and secondary sexual characters (Thampy and John ,1972 ,73; Nagamine *et al* ., 1980) and also behaviorial sexual characters (Charniaux - cotton , 1960 , 1961) .

Once the external male sexual characteristics are formed in gonochoristic shrimp , the androgenic gland is not needed for their maintenance (Fouir , 1997) . The role of androgenic gland in the regulation of the development of external sexual characters in decapods is well established. . It was recently demonstrated by injecting the cray fish *P . clarkii* with androgenic gland extracts , that the appearance of the external male characters is affected in the form of reversed spines . Injections of sperm duct extracts on control produced no such effect (Taketomi *et al* ., 1990) .A strong correlation was also found in *P.clarkii* males between the development of the androgenic gland and the morphological differentiation of the abdominal appendages (Taketomi *et al* ., 1996) . Masculinization of the external characteristics of female cray fish was observed after the implantation of androgenic gland (Nagamine and Knight , 1987) . A high degree of feminization , which included initation of oogenesis and development of oviducts and female gonopores , occured in maturing *M . rosenbergii* males that had been andrectomized in the early developmental stage . Males andrectomized in later developmental stages were either partially feminized or not feminized at all (Nagamine and Knight *et al*., 1980 a). Reimplantation of the androgenic gland into andrectomized *M . rosenbergii* males reversed the effect of the andrectomy . Androgenic gland implantation masulinized female recipients , as manifested by the development of the appendices masculina , the male gonopore complex , mature masculine chelipeds and initiation of spermatogenesis in the ovaries (Nagamine *et al* . , 1980 a , 1980 b) . A wide range of abnormalities in gonadal development was observed in andrectomized males , depending on the age at which the andrectomy was performed . Development of reduced testis was observed in males andrectomized at a relatively old age .In younger andrectomized males there developed testicular and partly ovarian gonads ("ovotestes") or abnormally lobulated ovaries (Snir, 1992). Sagi and Cohen (1990) observed complete sex reversal as a result of surgical removal of the androgenic gland in juvenile *M. rosembergii*,leading to the development of functional females capable of mating and producing progeny . Functional sex

reversal of female *M . rosenbergii* by implanting androgenic glands into the youngest and smallest prawns that would be identified as females has been reported by Malecha *et al* . (1992) . In both cases , progeny was obtained when fertile sex reversed animals were crossed with normal prawns , and the sex ratio of the offspring supported the homogametic male theory , in keeping with Katakura (1989) .

The androgenic gland , enlarges as and when there is an increase in activity . The increase in biomass of the androgenic gland in the case of *M. idae* was reported to be achieved by increase in length by Thampy and John (1973) . Further , these authors also noted that when there is an increase in the length of chelipeds , appendix masculina and the width of vas deferens , there is a corresponding increase in the size of the androgenic gland . This amply demonstrates that androgenic gland in malacostracan crustaceans is controlling determination of sex as well as regulation of primary and secondary sexual characters (Sagi , 1988) .

It was demonstrated (Sagi *et al* ., 1990) that androgenic gland ablation affects growth rates and morphotypic differentiation in the three distinctive adult male morphotypes that co - exist in *M . rosenbergii* populations (Kuris *et al* ., 1987 ; Sagi and Raa'nan , 1988) . Andrectomy of small males (SM) did not prevent transformation into the orange claw (OC) morphotype but did prevent further transformation into the blue claw (BC) morphotype . The growth rate of the andrectomized small and orange claw males was significantly lower than those of the control prawns (Sagi ,1990) . In *M.rosenbergii* the male growth rate was considerably higher than the female rate (Sagi *et al* ., 1986) . The somatic growth of andrectomized males was significantly lower than that of Sham - operated and normal males and very similar to that of normal females . In all the morphotypes andrectomy results in decreased growth rate (Sagi *et al* ., 1990) and hence an influence of androgenic gland on somatic growth is suggested (Kuris *et al* ., 1987) .

The androgenic gland undergoes hypertrophy after eyestalk ablation in several decapods (Adiyodi and Adiyodi , 1970) . Hyperactivity and hypertrophy of glandular tissue and precocious spermatogenesis in destalked crabs suggest that the androgenic gland is under the direct neurohormonal inhibitory control of the x - organ sinus gland system of the eyestalk (Demeusy and veillet , 1958) .

2.4.1.1.1. The androgenic gland , intersexuality and early sex differentiation

Intersexuality in gonochoristic decapods may shed some light on the role of the androgenic gland in early sex differentiation . The expression of intersexuality in *Cherax quadricarinatus* in

which one half has male internal characters and the other female characters, while the secondary external characters are masculine on both sides (Sagi *et al.*, 1996) conforms with the hypothesis that early male differentiation in decapods is mediated by a secretion from the androgenic gland primodium which diffuses along the genital tract (Charnianx - cotton and Payen , 1988) . This may explain the presence of the male reproductive system and the absence of the female system on the side , on which the androgenic gland exert its local effect through diffusion . On the other side , in the absence of an androgenic gland , differentiation of ovary is permitted (Charnianx - cotton , 1959 ; Sagi *et al.*, 1996) . Contradictory findings were recorded by Nakamura and co-worker (1992) who studied the time schedule of organogenesis of the genital organs and developments of androgenic gland in *Penaeus japonicus* . These studies suggested that the androgenic gland is inactive during organogenesis of the genital organs , leading to their conclusions that the differentiation of the testis is induced genetically , without the participation of the young androgenic gland . These authors also suggested that some other factor (from the ejaculatory bulb) is responsible for male differentiation of the primordial gonad (Nakamura *et al.*,1992) . A brain factor was suggested (Touir , 1977) to be responsible for male differentiation based on reports from other decapods (shrimp and crab) that the maintenance of the testicular germinal zone , sperm duct and androgenic gland is dependent on a brain factor.

2 .4 . 1 . 1. 2 Anatomy and histology of the androgenic gland in decapods

In decapods the gland is usually located at the subterminal portion of the sperm duct . The cells may be arranged as thin , parallel and anastomosing cords (Carpenter and De Roos , 1970 , Thampy and John 1972 , 1973) or in a compact lobed structure (kleinhotz and Keller , 1979) . A combination of the two structures was found in *M . rosenbergii* in which the androgenic gland is composed of strands of cells surrounded by a thin layer of connective tissue , forming a pyramidal cluster loosily associated with the posterior portions of the ejaculatory duct(Veith and Malecha,1983). Veith and Malcha (1983) observed three principal cell types in the androgenic gland of *M . rosenbergii:* type 1 cells are small with a dense cytoplasm , often containing two nuclei ; type 2 cells are slightly larger and vacuolated ; and type 3 cells are large , with vaculoes filling most of the intracellular space. Certain areas of the gland display cellular degeneration , possibily indicating a holocrine mode of secretion . The androgenic gland of *M . rosenbergii* stained positive for lipids , of which the highest concentration was found both in the gland and the epithelial cells lining the lumen of the ejaculatory duct . The lipids appeared to be evenly distributed throughout the gland and was not confined to any of the three cell types (Veith and Malecha, 1983). Thampy and John (1973) pointed out that the extent of disintegrating tissue is considerably greater in

the animals with long chelipeds in the case of *M . idae* . This suggests that a greater amount of the androgenic hormone is being liberated into the body fluid of animals with hypertrophied sex characters .

2 . 4 .1.1.3 Regulation of the androgenic gland

Sex and reproduction are neuroregulated by hormone from the central nervous system . Eyestalk neuropeptides such as gonad - stimulating hormone (GSH) and gonad - inhibiting hormone (GIH) apparently act directly on the ovaries (Charniaux - cotton and Payen, 1988 ; Quackenbush , 1991 , Fingerman , 1995) , whereas in males their action on testes appears to be indirect via a direct effect on the androgenic gland (Adiyodi , 1984 ; Gupta , 1989 ; Hasegawa et al ., 1993) . Bilateral destalking of the protandric shrimp *Pandalus platyceros* caused an increase in RNA synthesis in one particular cell type (the C cell type) in the androgenic gland (Brockenbrough -Foulks and Hoffman , 1974). Based on experiments with bilateral eyestalk ablation and eyestalk extract injection , Kulkarni *et al* . (1984) concluded that hormone released from the neuroendocrine system regulates androgenic gland activity . Sarojini *et al* . (1994) presented the hypothesis that in *P . clarkii* serotonin stimulates the release of GSH , which in turn acts upon the androgenic gland , which releases the androgenic hormone .

2.4.1.2 Females

In the case of females it is the ovary which secretes a hormone that regulates the female secondary sexual characters as has been proved by overiectomy in early stages which is known to prevent the secondary sexual characters from developing (Charniaux - cotton , 1960) . The development of temporary secondary sexual characters such as breeding dress (egg bearing hairs) seems to be conditioned by the amount of yolk deposited in the ovary , as reported in *Leander spp* (Callen , 1940) and *Caridina nataraJini* (Thampy , 1972) . In the case of *C. nataraJini* (Thampy , 1972) noted that females which had undergone spawning moult , but not provided with a male , resorbe the yolk by eighth day after spawning moult . If the resorption is complete the breeding dress is lost during the moult at the eighth day after spawning . In destalked females this process of resorption was found to be little slower than normal ones resulting in some yolk remaining to be resorbed and in such females the breeding dress is partially retained . This partial retention of breeding dress in the case of females undergoing resorption of yolk brings in a better correlation between the quantity of yolk in the ovary and the degree of development / retention of the breeding dress .

The overaictomy of the females during the reproductive period is known to be always followed by complete replacement of the ovigerous hairs by juvenile hairs and implantation of ovary results in the yolk deposition and appearance of ovigerous hairs in *Orchestia gammarella* (Charniaux -cotton ,1960). These observations clearly indicate that the permanent and temporary secondary sexual characters in female crustaceans are under the control of ovarian hormones .

2.4.2 *Endocrine control of reproduction*

A crustacean gears its reproductive activity in such a way that the liberation of young takes place when conditions are optimal for survival of young . This adaptive synchrony is a part of the individuals genetic endowment , which expresses itself via nervous- , endocrine and neuroendocrine channels . Various extrinsic cues , such as changes in day length , availability of food , fluctuations in temperature and proximity of prospective mating partners , are noted by sensory receptors , the resultng afferent nervous impulses converge on the central nervous system (CNS) , which in turn sends directional messages to the concerned organs , thereby eliciting specific response such as promotion or suppression of gametogenesis , uptake of vitellogenin , or sexual receptivity (Adiyodi , 1985) .

The reproductive biology is central to all biology and reproduction like many other physiological processes is under endocrine control in both invertebrates and vertebrates (Adiyodi , 1980) . The eyes in decapods are generaly stalked and movable and eyestalk is known to contain a variety of hormones or factors apparently governing such diverse functions as growth , moulting metabolic rate ,heart rate , metabolism of sugars and proteins , water balance , dispersion of pigments and sexual activity (Lockwood , 1968) . The X-organ Sinus gland (XO - SG) complex in the eyestalk is beleived to produce a hormone controlling both reproduction and moulting (Adiyodi and Adiyodi , 1970) . Two hormones jointly involved in the development have been postulated later to regulate reproduction jointly (Adiyodi , 1980) .

Although many observations have been made on the inhibition of reproductive maturation by eyestalk hormone (s) , recent research has focused mostly on brain , thoracic ganglion (TG) , ovary and mandibular organ (MO) and their functions are closely related with the release of gonad stimulatory factors or hormone (s) (Yano , 1992) .

As with ovarian maturation , it has long been suspected that vetellogenesis in crustaceans is controlled by two antagonistic hormones in penaeid shrimp, GIH which is secreted from the

XO - SG complex inhibits vetellogenesis and GSH secreted from the TG and brain stimulates vitellogenesis (Yano , 1992).

2.4.2.1 Target gland control

Dall *et al* . (1990) proposes a target gland control of reproduction in crustaceans . This target gland control is via GIH and its reduction either natuarally or by eyestalk ablation (ESA) , permits the target gland to function fully . This removal of GIH inhibition on the gonads permits them to develop to maturity . This system is identical to the optic gland of cephalopod molluscs and corpora allata (CA) of insects (Adiyodi and Adiyodi , 1970) . But this system is different in that target gland hormones in other animal groups are mostly stimulatory (for eg :vertebrate brain hormone and insect brain hormone).

2.4.2.2 Gonadal maturation

There is still much speculation and divergence of opinion about the process of gonadal maturation and the model of Adiyodi and Adiyodi (1970) is still current (Adiyodi 1985) . This scheme proposes that the actions of moulting hormone (MIH) and gonad inhibiting hormone (GIH) are antagonistic and also there is a gonad stimulating hormone (GSH) , produced by the brain and the TG . Moulting occurs when the titres of MIH and GSH are low and those of GIH and moulting hormone (s) , MH are high , gonad maturation occurs in the converse situation . This model applies primarily to the females , but there is evidence that in male crabs , GIH acts via the androgenic gland (AG) by inhibiting its secretion ; in its absense maturation takes place (Payen *et al* ., 1971) . In species where moulting and ovarian development alternate , MIH and GIH must act antagonistically (Dall *et al*., 1990). Moulting and reproduction are generally held to be antagonistic events in malacostracan crustaceans since both require large amounts of energy and are mechanically incompatible (Anilkumar and Adiyodi , 1987 ; Quackenbush and Herrnkind 1981 ; Chang , 1984 , 1992) .

2.4.2.3 Inhibitory factors of gonad maturation

2.4.2.3.1 Gonad inhibiting hormone (GIH)

In decapods , it is well known that the removal of eyestalk (ES) induces ovarian activity (Adiyodi and Adiyodi ,1970) . The existence of a gonad inhibiting principle in the evestalk of

decapod crustanceans was first demonstrated about 50 years ago by Panouse (1943) with his observation of accelerated ovarain growth in eyestalk ablated female shrimp , *Palaemon serratus* . The neuro endocrine complex produces an inhibiting hormone . When this is removed precocious gonadal development ensues . This was later confirmed in many decapod crustaceans , Stephens , (1952) in (*'amharus* , Brown and Jones , (1949) in *Uca* , Carlisle , (1953) in *Lysmata* , Demensy and Veillet , (1952) in (*'arcinus*) including many palaemonids and penaeids . Panouse (1944 , 1946) further found that removal of the sinus gland (SG) alone leads to some increase in size of ovary , but not nearly so great an increase as after eyestalk ablation (SA) . The effect of ablation of ES or removal of SG on the ovary can be prevented entirely (Pamouse , 1944 , 1946 ; Carlisle , 1953) or partially (Brown and Jones , 1949 ; Rangreker and Deshmukh , 1968) by implanting SG tissue into the operated animals . Injection of extracts of whole ES , or SG alone or the XO (GXO) alone , prevents any increase in ovarian weight after ES removal (Carlisle , 1953) , whereas injection of extracts of other fractions has no such effect nor has oral administration of SG after ESA (Knowles and Carlisle ,1956) . In intact animals the normal increase in ovarian size which preceeds the breeding season may be inhibited by injection of extracts of whole ES or SG or medulla terminalis GXO - MTGX - (Carlisle , 1953) . Knowles and Carlisle (1956) took these results as evendence for existence of an ovary inhibiting hormone (OIH) . ESA according to them removes an inhibitor which is preventing ovarian growth , thus leading to rapid uninhibited proliferation of the ovarian tissue which may increase several fold in a month. Conversely injection of ES extract (ESE)supplies the inhibitor which keeps the ovary in check .

Though Carlisle (1954) proposed specificity of GIH , Knowles and Carlisle (1956) , Payen *et al* . (1967) and Juchault and Legrand (1967) , argue that OIH and TIH are not different from one another and termed the inhibitory hormone in both sexes as GIH . Quackenbush (1991) agrees to this arguement and Chang (1992) finds little reason to suspect that OIH and vitellogenesis inhibiting hormone (VIH) mentioned by various workers are different from that of GIH . GIH appears to be present not only in adults , but in inmature stages as well ; in *Potamon dehaani* , ES of inmature crabs of both sexes have been stated to contain the hormone (Otsu , 1963) . When vitellogenesis is already in full swing ESA does not perceptibly accelerate ovarian growth suggesting that during this period the synthesis of GIH and / or its release from ES into general circulation may be very low , the possibility that this may be related to an optimum is not ruled out (Adiyodi and Adiyodi , 1970)

Many workers suggest that GIH is produced by the SG - XO complex in alternation with a MIH (Laufer and Landau , 1991 ; Yano , 1992) . In adult female of several species ESA results not in moulting , as in juveniles and some adults of some decapods , but in premature yolk deposition

in the ovary , both during non - breeding season and in certain species like *Paratelphusa hydrodromous* (Gomez , 1965) and *Scylla serrata* (Rangneker and Deshmukh , 1968) even in prepubertal stage . Thampy (1972) also holds the view that there is only one hormone which acts at the metabolic level . Adiyodi (1980) suspects that MIH and GIH represent a single hormone say growth restraining hormone (GRH) that exercises its influence on two target processes ,viz ,growth . and reproduction .Crustacean eyestalk contains hormone that inhibits moult and reproduction , but the course of events initiated by ESA varies with species , age of individual , season and other factors (Adiyodi , 1985 ; Quackenbush , 1986 ; Fingerman , 1987).

Primary action of GIH in females apparently occurs during the secondary vetellogenesis , the time when ovary increases dramatically in size due to synthesis and uptake of yolk proteins produced in either follicle cells or extra ovarian sites (Quackenbush , 1991 ; Chang , 1992) . But in *P . hydrodromous* there is evidence to show that GIH principally inhibits the primary vetellogenensis in these crabs , however, its effect on secondary vetellogenesis is far from impressive(Kurup and Adiyodi , 1980) . Alternatively GIH may have non - ovarian target or in fact there may be more than one ES factors which inhibit ovarian growth (Laufer et al ., 1992) .

GIH , eventhough inhibitory in high titres to ovarian growth , is necessary in declining titres to ensure the proper development of the ovary (Adiyodi and Adiyodi , 1970) . GIH is therefore not a GIH in its true sense but only a gonad restraining hormone (Thampy 1972 ; Adiyodi , 1981) . Anilkumar and Adiyodi (1986) found that removal of GIH by ESA may induce some precocious ovarain development , but such development was not normal .

Kallen and Mensy (1989) have advanced the theory that GIH is similar in structure and not different from crustacean hyperglycemic hormone (CHH)

2.4 .2.3.2 Other Inhibitory factors

The androgenic gland which is responsible for the masculinisation of the animal seems to produce a number of compounds including farnesyl acetone, a molecule similar in structure to methyl farnesoate (MF) (Ferzon *et al* ., 1978) and this will inhibit ovarain lipovitellin synthesis in vitro (Bessuer - bonnefaunt and Lawrence , 1984) .

It is well known that biogenic amines release peptide neurohormone from neuroendocrine structures in several crustaceans(Fingerman , 1985) . Certain biogenic amines (Octamine and Serotin) inhibited MF synthesis in *Libina emarginata* (Mancola *et al* . , 1989).Laudan *et al* . (1989)

found that pigment dispensing hormone (PDH) significantly inhibits mandibular organ synthesis of MF in *P . Clarkii* . Quackenbush and Hernkind (1983) reported that patially purified GIH could not be seperated from PDH .Thus in some cases the functions of pigment dispersal and gonad inhibition may be performed by the same or similar molecules (Laufer *et al* . , 1987).

2.4.2.4 Stimulatory factors for control of reproduction

2.4.2.4.1 Gonad stimulating hormone (GSH)

A second reproductive neurohormone is found in the brain and TG which acts to stimulate ovarian development in shrimps , crabs and lobsters . The concept of ' bihormonal system ' was first proposed by Otsu (1960 , 1963) . Otsu (1963) after working with *Potamon dehaani* and Yano and Wyban (1992) with *P . vannamei* suggested its existence because ESA caused precocious overian growth in adult , but not in juveniles . This lead them to reason that not only was the absence of GIH required for ovarian growth , but the presence of a stimulatory hormone is also necessary . Otsu(1963) also observed that implantation of adult TG was effective in triggering maturation of ovary in ESA juveniles . The experiments of Hinsch and Bennett (1979) using *L . emarginata* , Gomez (1965) using *P . hydrodromus* with both brain and TG proved that GSH from TG has got a role to play in ovarian maturation . Extract of TG of reproductive *Uca pugilator* stimulates ovarain growth in adult crabs (both intact and ablated) while TGE from non - reproductive crabs has no effect on normal crabs and had actually inhibited ovarain growth in ablated crabs (Eastman - Reks and Fingerman , 1984) .

Implantation of brain and TG into the male *Paratelphusa hydrodromus* resulted in precocious maturation of the testis and even a hypertrophy of the vas deferens (Gomez , 1965) . This observation together with the finding of Otsu (1963) that the TG implantation effectively accelerated ovarian development in young female *Potamon dehanni* and the experiment of Yano *et al* . (1988) where the TG implantation of mature female *H . americanus* into *P . vannamei* induced ovarian growth , suggests that GSH which is effective in both sexes in different genera is present in TG and perhaps also in the brain of crabs and shrimps .

The role of GSH appears to be dual in that it promotes oocyte growth and prevents Y -organ (YO) activity ; the latter accomplished either directly or indirectly by raising the level of MIH and / or lowering the level of GIH (Adiyodi and Adiyodi , 1970).

2.4.2.4.2 Juvenile hormone

The role of terpenoid hormone unique to arthropods , and collectively known as JHS or juvenoids has been established in insect reproduction (Raabe , 1982 ; Laufer *et al* ., 1992) . The JHS appears not only in development of insect larval stage but also in the regulation of reproduction (Downer and Laufer , 1983) . In recent years , attention has been focussed on another gland , the Mandibular Organ (MOs)as a source of gonad stimulating factor in decapod crustaceans (Subramoniam and Kellar , 1993) . Since both the arthopod sub - phyla , the Insecta and Crustacea , are already known to regulate moulting with identical hormones , 20 - hydroxyecdysone (Karlson , 1956 ; Hampshire and Horn , 1966 ; Laufer *et al.*, 1987) , it is speculated that the Crustacea might also have a functional JH for development and reproduction (Laufer et al ., 1992 ; Chang et al ., 1992). This view is supported by considerable literature . There are reports of insect JH or related compounds having biological activity in Crustacea and of crustarean tissues having JH activity in insects . The MF , the immediate precursor of the insect JH III , has been showed to be present in several decapod crustacean species (Laufer *et al* . , 1986) . In addition , the MO of decapod crustaceans is structurally similar to Corpora Allata (CA) of insects (Le Roux , 1968 ; Byard *et al.*, 1975) . If MO is a structural homologue of CA , it is reasoned (Laufer *et al.*, 1987) that it may produce one of the JHS . More interestingly , the activity of MO appears to be controlled by ES , as evidenced by the finding that ESA results in the hypertrophy of this gland (Le Roux , 1983 ; Laufer *et al.*, 1986) . Farnesoic acid (FA) , a precursor to MF is also secreted in large amounts by MO in mud crab such as *S . serrata* (Tobe *et al.*, 1989) . Wherever the gland was found , its activity always appears to be significantly related to the reproductive state of the organism (Laufer *et al* ., 1986 ; Borst *et al.*, 1987) .

2.4.2.4. 3 Steroid hormones

Steroid hormones have been localised by several methods in many crustacean tissues(Skinner , 1985 ; Fingerman , 1987) . Steroid hormones other than the ecdysone have been found in crustacean eggs , ovarain tissue and the MO (Adiyodi , 1985 ; Couch and Hagino , 1983) . The location of these steroid hormones , progesterone and estradiol suggests that they may have a role in regulation of reproduction in crustaceans (Quackenbush , 1991 ; Young *et al.*, 1992) .The moulting hormone (MH) , ecdysone is known to play a role in insect reproduction and therefore may act in a similar fashion in custaceans (Laufer and Landan , 1991 ; Laufer *et al.*, 1992) . Y - organ seems to play a role in the endocrine control of gonadal function , for its ablation in either sex in crabs

before the onset of sexual maturity leads to a considerable retardation of gametogenesis and degenarative changes in the gonads (Echalier , 1954 ; Arvy *et al.*, 1956) . Though observations of many workers indicate that MH is not essential for reproduction , but only high titre of MIH (Adiyodi and Adiyodi , 1970) , extirpation of YO from the breeding and juvenile females results in an acceleration of vitellogenesis in *C. maenas* (De'meusy , 1963) .

There is now a growing body of evidence to suggest that in insects and crustaceans , ecdysteroids (ECD) which are primarily MH (Adiyodi and Adiyodi , 1970) are also there in adult life to stimulate the ovarian growth (Adiyodi , 1980) . Prepubertan growth and development of gonads appear to be part of the normal genetically determined growth process and ECD may have a role in post pubertal development (Adiyodi , 1985) . The MH may mediate several different aspects of crustacean reproduction and development . There are reports that ECD may be involved in vitellogenesis since it was isolated from the ovaries , in relatively high concentration (Lachaise *et al.*, 1987 ; Adiyodi and Subramoniam , 1983) . It seems reasonable to suggest that ECD functions primarily as MHs in crustaceans and the effect that it may have on reproduction , may be indirect or secondary (Adiyodi , 1978) Recent discovery of the presence of ECD in crustacean ovary suggests that the crustacean gonads may secrete or accumalate active hormone principle (Adiyodi , 1985 ; Chang , 1991 ; Young *et al.*, 1993) .

Evidence is accumalating none the less , from scattered works suggesting that crustacean ovary might play a role in the biosynthesis of steroid hormone (s) . Estrogen was detected in the ovaries of *Panulirus argus* and freshly spawned eggs of the lobster , *H.americanus* (Donahue , 1940 , 1948 , 1952 , 1957) . Lisk (1961) confirmed this estrogenic compound to be 17 - β estradiol. Kanazawa and Teshema (1971) detected progesterone and testosterone in the ovaries of *Panulirus japonicus* . A number of steroids including testoslerone , progesterone and pregnenolone have been identified in the gonads and serum of the crayfish *Astacus leptodactylus* and the lobster . *H . americanus* (Burns et al., 1984 ; Ollivier *et al.*, 1986) and the shrimps *P .monodon* (Young *et al.*, 1992) and the crab *C . maenas* (Hazel , 1986) .

2 .5 Methods of inducing maturation in prawns

Securing of ripe spawners from wild is costly and uncertain.This has generated interest in the induced matuation of shrimps under controlled conditions . Development and management of shrimp broodstock is now an integral part of the hatchery (Muthu and Laxminarayana , 1952 ; Muthu,

1983) . Male shrimps generally mature in captivity so that induced maturation mainly concerns females and so studies on reproduction have predominently focused on female maturation (Prima vera , 1985 , 1988) . There are three basic approaches employed singly or in combination to induce ovarian maturation in penaeids - endocrine , environmental and nutritional (Primavera , 1985) .

2.5.1 *Environmental*

It is apparent from vast literature available on environmental monitoring of reproduction that salinity , light , temperature and pH are of paramount importance for successful reproduction of crustaceans (Bouchon , 1991) . Crustacean breeding may synchronize with a combination of environmental factors , the relative importance of which may vary in different species and in different environments (Bouchon *et al.*, 1992) .

2.5.2 *Nutritional method.*

Nutrition is profoundly important for reproduction and success of reproduction closely relates to nutrient ingestion accompanying ovarian development (Bray and Lowrence , 1992) . Live or fresh frozen components used are expensive , may deteriorate water quality and may vary in nutritional quality with species, age , maturation state , season and location (Bray *et al.*, 1990) . Brood stock are generally fed *ad libitum* with fresh and frozen clam (Muthu and Laxmi narayana, 1979) , squid meal (Aquacop ,1975 ; 1977 ; 1979) marine worms (Ponnuchamy *et al.*, 1981) shrimp(Beard *et al.*, 1977) , fish (Halder , 1978 , 80) , supplimented with dried pellets (Moore *et al.*, 1974 ; Aquacop , 1977 , 79)

The effect of ascorbic acid on the sperm and spematophore quality of *P . vannamei* has been recently documented (Leung - Trujillo and Addison , 1990) . Generally , diets containing artificial and natural food give the best results (Galgani *et al.*, 1989) .

2.5.3 *Endocrine methods*

2.5.3.1 Use of exogenous hormones

Crustacean gonads secrete steroids more usually identified with vertebrates and also possess the enzymatic capacity to synthesize vertebrate sex steroids (Burns *et al.*, 1984). Donahue (1940 ; 1948) found estrogen in the ovary of *Panilurus argus* and showed that testis of

Homarus americanus contains testosterone . Human chorionic gonadotropin stimulated oogenesis of sand shrimp *Crangon crangon* (Bomirski and Kelk , 1979) .

The androgenic gland of testosterone injected *Parapenaeopsis hardwickii* and *P . stylifera* (Nagabhushanam and Kulkarni , 1981 ; Naghabhushanam *et al.*, 1987) showed hypertrophy and hyperplasia .

2.5.3.2 Eyestalk ablation

Eversince the observation of Panouse (1943) that ESA leads to ovarain growth in sexually quiescent *Palaemon serratus* , ESA has been used as a tool by several workers to induce maturation and spawning in many species of shrimps and prawns .

In the eyestalk of decapod crustaceans a gonad inhibiting hormone is produced by the neurosecretory cells of x-organ and transported to the sinus gland for storage and release . Thus ESA destroys XO - SG complex and leads to either precocious moulting or gonadal maturation depending on the interactions of ambient temperature and age of animal (Adiyodi and Adiyodi , 1970) . Except for a few studies , endocrine manipulation has been synonymous with eyestalk ablation , a technique first performed in the penacid shrimp , *P . duorarum* by Idyll (1971) and Caillout (1972) with far reaching impact on aquaculture (Primavera , 1985) . Now the process of unilateral eyestalk ablation is used in almost all the shrimp and prawn maturation or reproduction facilities in the world , both research and commercial , to stimulate female shrimp and prawn , to develop mature ovaries and to spawn (Bray and Lawrence , 1992) .

Destalking has been accomplished through different methods . The simplest , method is to cut the eyestalk near its base with a pair of sharp scissors (Arnstein and Beard , 1975 ; Lumure , 1979) . However this leads to profuse bleeding in delicate species and results in high mortality . Calliouet (1973) performed ablation by cutting the eyestalk near the base with the help of sharp scissors and the wound cauterised immediatly with a pencil type soldering iron to avoid loss of haemolymph . Muthu and Laxminarayana (1979) used medical electrocautery apparatus to accomplish destalking ; such a method resulting in simultaneous cutting and sealing of the wound , thus ensuring 100 % survival of the destalked animals .

The high mortality and inability to spawn after bilateral removal of eyestalk prompted researchers to abandon the method of bilateral eyestalk ablation (Muthu and Laxminarayana , 1982).

Temporal synchronization of eyestalk ablation with moult cycle is an important factor for the production and synchronization of egg production and related reproduction (Emmerson,1980). Ideally , ablation should be undertaken during intermoult for maturation to follow , during premoult it leads to moult and subsequently longer latency period (Aquacop , 1979 ; Primavera , 1979) and during postmoult to mortality, because of added stress and excessive loss of haemolymph (Aquacop , 1979) . Animal ablated early in the moult cycle get enough time between ablation and spawning (Browdy and Samocha ; 1985) .

Eyesalk ablation in males has received little attention despite the demonstration of male ablation yielding increased gonad size and precocious development (Adiyodi and Adiyodi,1970; Young , 1974 ; Lawrence *et al*., 1979).Researchers have considered maturation of female prawns as the major limitation in reproduction in captivity as the presence of sperm and spermatophore throughout the year has been reported (King , 1948 ; Pillay and Nair , 1971 ; Santiago , 1977) . However , a pronounced seasonal cycle of male gonad size has been observed in *Metapenaeus affinis* (Pillay and Nair , 1971).

Spermatophore production seems to be controlled by x -organ sinus gland complex . Ablated *P . setiferus* produced new spermatophore almost twice as unablated males (Leung - Trijillo , and Lawrence , 1991) .

Ablated *P . vennamei* produced larger spermatophore with more than two fold increase in sperm count over controls with no apparent effect on sperm quality (Lewing - Trijillo and Lawrence,1985) . Bilateral ablation further enhanced gonad weight , spermatophore weight and spermatophore count with the exception of sperm count (Leung - Trijillo and Lawrence , 1985) . Chamberlian and Lawrence (1981) reported a high mating frequency in tanks containing unilaterally ablated males, compared to unablated *P . vennamei* . These observations suggest the usefullness of eyestalk ablation in males also .

2 . 6 Allometric growth pattern in different sexes

In crustaceans there is allometry in growth between the sexes and among different phases of growth within each sex. In some instances these differences in allometry level are relatively small and of uncertain significance as when two carapace dimensions are compared , or when the length of the walking legs is compared to the length of the carapace . But there are organs which show

striking and consistant patterns of variation in the level of allometry , notably the chelae . Enlarged chelate or subchelate appendages occur in many groups such as Tanaidaceans and Decapods .

In females , the level of allometry in chela growth is generally near unity in all phases , so that growth is essentially isometric throughout . There are only minimal changes in allometry level at the pre - puberty and p uberty moults and not significiant change in relative size at prepuberty moults . In males , growth tends to be isometric in the undifferentiated phase, but the level of allometry increases at the pre - puberty moult so that the juvenile phase has positive allometry . At the puberty moult there is usually a further marked increase in the relative size of the chela .

Kuris *et al* . (1987) found that the level of allometry varies among different male morphotypes of *M* . *rosenbergii* .The BC males are readily distinguished from other male morphotypes, having greater propodus and carpus length in relation to carapace length , the carpus of a BC male averages 61% longer than that of an OC male of similar size and the difference between these males in terms of propodus length is 47 %. They found that within BC morphotype , growth of the carpus is nearly isometric . Comparing the length of chelipeds they found that the cheliped length of BC males is unequivocally greater than for other males of similar body size and total cheliped length showed positive allometry . They also noted that the BC males were readily distiguished from other morphotypes with respect to propodus width . The propodus of BC males was relatively narrower than the propodus of other male morphotypes .

In *Macrobrachium* , the level of development of male sex charecters is directly related to the length of the androgenic gland but not related to the body size of the prawn . Thampy and John (1973) found that in *M*. *idae* the length of the cheliped , the length of the appendix masculina , number of spines on the appendix masculina and the width of vas deferens had a significiant correlation with the length of the androgenic gland but not with the size of the prawn .

Kuris *et al* . (1987) reported that maturation of *M* . *rosenbergii* from juvenile male lacking appendix masculina , to the small male morphotype is not accompanied by an allometric change in claw segments .

3 . MATERIALS AND METHODS

3.1 Source of animals

Macrobrachium idella of almost equal sizes were collected from Panagad area of Cochin backwaters near the College of Fisheries , Cochin during the period July- September, 1997. Animals were maintained in 1 ton fibre glass tanks kept in *Macrobrachium* hatchery of the College of Fisheries. Aeration was given from an air compressor through an airstone placed in the centre of the tank. The day time temperature ranged from 28-30 degree celsius .

3.2 Feeding

The animals were fed daily with clam meat . The excess feed was siphoned out daily .

3.3 Eyestalk ablation

Eyestalk ablation was effected in the early hours of the day using an electrocautery apparatus after dipping the prawns in icewater ,following the method of Muthu and Laxminarayana,(1979).

3.4 Experimental procedure

3.4.1 *Study to find the relationship of body size with the androgenic gland and the primary and secondary sex characters*

A random sample of 30 males from the collection made during July - Aug was taken . The body size , the size of the cheliped and appendix masculina together with number of spines were noted . These specimens were then dissected out and the size of the androgenic gland and width of vas deferens were noted to find out the relationship of the size of the androgenic gland with that of cheliped length , spinousity of appendix masculina , width of vas deferens and the body size .

3.4.2 *Experiment to find out the effect of eyestalk ablation on development of androgenic gland and sexual character*

An experiment to study the effect of destalking on androgenic gland and sexual characters was conducted using 24 males collected during Aug - Sept. 12 males were destalked unilateraly while the other 12 served as control . The body length and cheliped length were noted . The

measurement of androgenic gland, width of vas deferens and length of appendix masculina was done from camera lucida diagrams of these after sacrificing and dissecting the prawns at the end of experiment . The experiment was carried out for a period of 45 days .

3.5 Analysis of water quality parameters

The following instruments / methods were used for the analysis of water quality parameters .

Salinity	:	Refractometer
Dissolved oxygen	:	Standard winklers method
Ph	:	PH meter
Temperature	:	Thermometer

3.6 Evaluation methods

3.6.1 Percentage gain in body length = $\frac{\text{(Length of prawn at the termination of the experiment)} - \text{(Length of prawn at the initiation of the experiment)}}{\text{Length of prawn at the initiation of the experiment}} \times 100$

3.6.2 Percentage gain in chela length = $\frac{\text{(Length of chela at the termination of the experiment)} - \text{(Length of chela at the initiation of the experiment)}}{\text{Length of chela at the initiation of the experiment}} \times 100$

3.7 Statistical design and analysis

In the first experiment to find out the relationship of the body size with androgenic gland and of primary and secondary sex characters , correlation analysis has been used .

In the second experiment to find out the effect of eyestalk ablation on development of androgenic gland and sexual characters , students ' t ' - test has been used for the comparison of ablated and control groups . Correlation methods have also been used for studying the relationship between different characters under the ablated and control condition . Angular transformation was effected to the percentage gain in body length and cheliped length before analysis .

4 . RESULTS

4.1 Study to find the relationship between the development of primary and secondary sex characters with that of androgenic gland

A study was conducted to find the relationship of the body size with the extend of the development of secondary sex characters such as second cheliped and appendix masculina , the primary sex characters viz the gonad and the development of the endocrine gland controlling the development of primary and secondary sex characters in the prawn , *M . idella* .

Data regarding the range , mean and SE of 30 male prawns collected during July - Aug is given in table no : 1

Sl No:	Characters	Range	Mean	Standard Error of Mean
1.	Length of prawn (cm)	7.8 - 9.2	8 . 517	0 . 378
2 .	Length of chelipeds (cm)	7.0 - 15.1	10 . 610	2 . 497
3.	Length of appendix masculina (mm)	2.5- 4.2	3 . 49	0 . 455
4.	No:of spines on appendix masculina	35 - 55	45 . 67	5 . 868
5.	Length of androgenic gland (mm)	3 - 5	3 . 83	0 . 628
6.	Biomass of androgenic gland (mm^2)	. 75 - 2.5	1. 456	0 . 075
7.	Width of vas deferens (mm)	. 76 - 1. 3	0 . 968	0 . 244

Table 1 Data on body size and of sex characters and androgenic gland of 30 male of *M.idella.*

Correlation analysis of the data collected from 30 prawns of varying sizes is given in table no : 2

characters	length of prawn	length of cheliped	length of androgenic gland	biomass of androgenic gland	width of vas deferens	length of appendix masculina	No: of spines on app. masculina
length of prawn	1	0.1791	- 0.0005	* 0.4093	0.0771	0.0390	0.0509
length of cheliped		1	0.1519	* 0. 3821	0.1942	0.0504	0.0814
length of androgenic gland			1	0.2722	* 0.3093	* 0.4236	0.0581
biomass of androgenic gland				1	-0.1230	0.2330	- 0.1913
width of vas deferens					1	** 0.6770	0.2560
length of appendix masculina						1	** 0.5641
No: of spines on appendix masculina							1

Table 2 Data on correlation analysis of body size and sex characters and androgenic gland of 30 males of *M.idella.*

Correlation analysis of the data collected from 30 prawns of varying sizes was done in order to understand the extend of relationship between various characters considered . Inspecting the correlation values it could be seen that some of the correlation values are significant (indicated by one asteric , $p \leq .05$) and some are highly significant (indicated by two asterics , $P \leq .01$) . High positive relationship was observed between width of the vas deferens and length of appendix masculina . Significant correlation could be found between biomass of androgenic gland and length of appendix masculina . Similar is the case with length of second cheliped and biomass of androgenic gland .

The results of the study clearly indicate that the development of secondary sexual characters such as second cheliped and length and spinuosity of appendix masculina and primary sex characters as indicated by the width of vas deferens are well correlated with the development of androgenic gland which is the endocrine gland responsible for the control of development of male primary and secondary sex characters in this prawn .

Data regarding the ratio between bodylength and cheliped length, bodylength and biomass of androgenic gland and bodylength and length of appendix masculina of 30 males are given below in table 3 .

Sl No	Length of prawn	Length of second cheliped	Length of App . masculina	Biomass of androgenic gland	Ratio of bodylength to cheliped	Ratio of bodylength to biomass of androgenic gland	Ratio of bodylength to app.masculina
1	8 . 5	11	3 . 5	1 . 52	1: 1 . 2	1: 0 . 17	1: 0 4
2	8 . 5	10 . 8	3 . 5	1 14	1: 1 . 2	1: 0 13	1: 0 . 4
3	6 . 5	7 . 5	2 3	1 22	1: 1 . 5	1: 0 . 15	1: 0 . 3
4	8 . 7	10	3	1 . 2	1: 1 . 1	1: 0 . 14	1: 0 . 3
5	8 . 5	10 9	3 . 7	805	1: 1 . 2	1: 0 14	1: 0 . 4
6	7 . 8	10 . 5	3 . 2	1 . 80	1: 1 3	1 0 10	1: 0 . 4
7	8 . 3	13 . 5	3 . 2	1 . 28	1: 1 6	1: 0 21	1: 0 . 3
8	8 . 2	10 5	3 . 5	1 28	1: 1 . 2	1: 0 . 15	1: 0 4
9	8 . 1	11	3 . 3	1 28	1: 1 . 3	1 0 . 15	1: 0 . 4
10	7 . 8	8	3 3	1 312	1: 1 . 02	1: 0 . 16	1 0 . 4
11	8 . 5	10 . 5	3 . 2	1 55	1: 1 . 2	1: 0 18	1: 0 . 3
12	9	15	4	2 . 5	1: 1 . 6	1: 0 . 27	1: 0 . 4
13	9	8	3 . 1	1 . 05	1: 0 . 8	1: 0 . 11	1: 0 . 3
14	9	12	4 . 0	1 . 88	1: 1 . 3	1: 0 . 20	1: 0 . 4
15	8 . 5	14	3 . 4	1 . 76	1: 1 . 6	1: 0 20	1: 0 . 4
16	9 . 2	15	3 . 5	1 . 71	1: 1 . 6	1: 0 . 18	1: 0 38
17	8 . 5	8 . 5	3 . 2	. 75	1: 1	1: 0 . 08	1: 0 . 37
18	8 . 6	8 . 5	3 . 5	. 9	1: 0 . 98	1: 0 . 10	1: 0 . 40
19	8 . 5	7	3 . 5	. 105	1: 0 . 82	1: 0 . 12	1: 0 . 40
20	9	7	2 . 5	. 9	1: 0 . 77	0: 0 . 10	1: 0 . 20
21	7 . 8	7 . 5	2 . 5	. 9	1: 0 . 96	1: 0 . 11	1: 0 . 32
22	7 8	8 . 5	4	2 0	1: 1 . 08	1: 0 . 25	1: 0 . 51
23	8 5	8 . 5	4	2 . 0	1: 1	1 . 0 . 23	1: 0 . 47
24	8 . 8	10	4 . 2	1 . 92	1: 1 . 1	1: 0 . 21	1: 0 . 48
25	9	14	4 . 2	1 . 76	1: 1 . 5	1: 0 . 19	1: 0 . 46
26	8 . 5	8 . 5	4	1 . 53	1: 1	1: 0 . 18	1: 0 . 47
27	7	15	4	2 . 5	1: 2 . 1	1: 0 . 35	1: 0 . 57
28	8 . 5	10	3 . 1	1 . 4	1: 1 . 2	1: 0 . 17	1: 0 . 38
29	8 . 1	15 . 1	4 . 2	2 . 16	1: 1 . 9	1: 0 . 27	1: 0 . 53
30	8 . 5	10 . 5	3 . 2	1 . 55	1: 1 . 2	1: 0 . 18	1: 0 . 37

It could be seen from table no:3 that there is no direct correlation between the bodylength and the secondary sex characters such as cheliped length, length of appendix masculina and number of spines on it. It could also be seen that individuals of same size, having cheliped length of different sizes, so also, the length of appendix masculina and the number of spines. It could also be seen that length and biomass of androgenic gland of males, also vary greatly in individuals having more or less similar body size.

The above data can be grouped into two, group A (Table .4) consisting of 22 individuals having a bodylength to cheliped length ratio below 1:1.5 and group B (Table .5) consisting of 8 individuals having a body length to cheliped ratio above 1:1.5 .

Table 4 . Ratio of body length to some sexual characters of 22 males where the bodylength to cheliped ratio is below 1 : 1 . 5 .

Sl No:	Ratio of bodylength to cheliped length	Ratio of bodylength to biomass of androgenic gland	Ratio of bodylength to length of App.masculina
1	1 : 1 . 2	1 : 0 . 17	1 : 0 . 4
2	1 : 1 . 2	1 : 0 . 13	1 : 0 . 4
3	1 : 1 . 1	1 : 0 . 14	1 : 0 . 3
4	1 : 1 . 2	1 : 0 . 14	1 : 0 . 4
5	1 : 1 . 3	1 : 0 . 10	1 : 0 4
6	1 : 1 . 2	1 : 0 . 15	1 : 0 . 4
7	1 : 1 . 3	1 : 0 . 15	1 : 0 . 4
8	1 : 1 . 02	1 : 0 . 16	1 : 0 . 3
9	1 : 1 . 2	1 : 0 . 18	1 : 0 . 4
10	1 : 0 . 8	1 : 0 . 11	1 : 0 . 4
11	1 : 1 . 3	1 : 0 . 21	1 : 0 . 38
12	1 : 1	1 : 0 . 08	1 : 0 . 4
13	1 : 0 . 98	1 : 0 . 10	1 : 0 . 2
14	1 : 0 .82	1 : 0 . 12	1 : 0 . 32
15	1 : 0 . 77	1 : 0 . 11	1 : 0 . 51
16	1 : 0 . 96	1 : 0 . 25	1 : 0 . 47
17	1 : 1 . 08	1 : 0 . 11	1 : 0 . 48
18	1 : 1	1 : 0 . 23	1 : 0 . 46
19	1 : 1 . 1	1 : 0 . 21	1 : 0 . 46
20	1 : 1	1 : 0 . 18	1 : 0 . 38
21	1 : 1 . 2	1 : 0 . 17	1 : 0 . 38
22	1 : 1 . 2	1 : 0 . 18	1 : 0 . 37

Table : 5 Ratio of body length to some sexual character of 8 males where the body length to cheliped ratio is above 1 : 1 .5

Sl No:	Ratio of bodylength to cheliped	Ratio of bodylength to biomass of androgenic gland	Ratio of body length to length of App . Masculina
1	1: 1. 5	1 : 0 . 15	1 : 0 . 3
2	1: 1. 6	1 : 0 . 21	1 : 0 . 4
3	1 1. 6	1 . 0 . 27	1 : 0 . 4
4	1: 1. 6	1 : 0 . 20	1 : 0 . 37
5	1: 1. 6	1 : 0 . 18	1 : 0 . 4
6	1 1. 5	1 : 0 . 19	1 : 0 . 57
7	1: 2. 1	1 : 0 . 35	1 : 0 . 57
8	1: 1 9	1 : 0 . 27	1 : 0 . 53

It could be seen that in group A (Table .4) , wherein the ratio of bodylength to cheliped length below 1:1.5, have their appendix masculina not fully developed and androgenic gland were not well developed, indicating that a direct relationship exists between the degree of development of androgenic gland to the extend of development of sex characters.

In group B (Table .5), wherein the ratio of bodylength to cheliped length is more than 1:1.5, there is higher ratio of body size to androgenic gland and also body size to length of appendix masculina, indicating that the increase in the length of cheliped is the result of increase in the biomass of androgenic gland.

In an animal (Sl . No : 27 , Table 3) body length is 7 cm and cheliped length is 15 cm, the ratio of bodylength to cheliped length is found to be 1 : 2 . 1 and ratio of bodylength to biomass of androgenic gland was found to be 1 : 0 . 35 , which are the highest .

In the prawn , wherein (Sl . No : 20 , Table 3) the lowest ratio of bodylength to cheliped length of 1 : 0 . 77 was obtained, has bodylength of 9 cm and cheliped length of 7 cm . The ratio of body length to biomass of androgenic gland in this case was found to be extremely low being 1 :0 .10.

4. 2 Effect of eyestalk ablation on bodygrowth , development of androgenic gland and development of male primary and secondary sexual characters

The data of the experiment to find out the effect of eyestalk ablation on androgenic gland and male primary and secondary sex characters conducted using 24 numbers of male *M. idella* of size ranging from 7-8 . 9 cm and cheliped length of 8 - 17 cm are given in the table no: 6.

TABLE 6

Sl No.	Characters	Initial			Final		
		Range	Mean	SE	Range	Mean	SE
1	Length of prawn (cm)	7 - 8.9	8 . 8666	0 . 476	8 - 9. 2	8. 9611	1 . 21209
		8. 3-9. 0	**8 . 5333**	**0 . 287**	**7 - 8 .9**	**8 . 2166**	1 . 64822
2	Length of cheliped (cm)	8 - 17	11 . 5416	2 . 358	9- 17.5	12 . 566	8 . 01914
		6.8-14	**8 . 725**	**1 . 945**	**7. 2 -14.1**	**9 . 0583**	6 . 21201
3	Length of App.masculina (mm)	--	--	--	2.3 - 3.8	3.2916	1 . 67082
					2.3 - 3.5	**2.7916**	1 . 09962
4	No : of spines on apppendix masculina	--	--	--	38 - 65	50 . 000	26 . 900
					30 - 44	**38 . 0833**	13 . 3750
5	Length of androgenic gland (mm)	--	--	--	4.0 - 5.5	4 . 5833	1 . 64822
					2 .8 -3.8	**3 . 2166**	0 . 96781
6	Biomass of androgenic gland (mm)	--	--	--	12-3 . 8	2.15	0 . 66458
					7-1 . 15	**.99**	0 . 14130
7	Width of vas deferens (mm)	--	--	--	.8 - 1.2	1 . 050	0 . 57445
					.5 - 1. 0	**0 . 7750**	0 . 56789

ablated
unablated

Table 7

Comparisons of the final percentage increase in length of body and cheliped length between ablated and nonablated *M.idella* males used for experiment to find out the effect of eyestalk ablation on androgenic gland and primary and secondary sex characters.

Sl No .	Parameter	Body length			Cheliped length		
		Mean	SD	t . value	Mean	SD	t. value
1	Ablated	13 . 7819	2 . 317	* 4. 2170	14 . 4115	6 . 0315	* 2 . 911
2	Non - ablated	9 . 1621	2 . 7972		7 . 8464	2 . 8102	

* Significant at 5% level

It could be seen from table no:7 and figures no:1 & 2 that there is a significant increase in the bodylength and cheliped length of those prawns which were unilaterally destalked .

Fig no:1 showing mean and standard deviation of final percentage increase in body length between ablated and unablated prawn, *M. idella* used in the experiment to study the effect of eyestalk ablation on androgenic gland and primary and secondary sex characters.

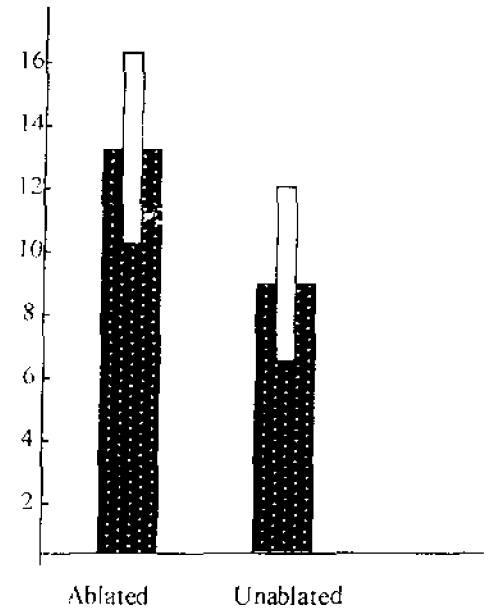
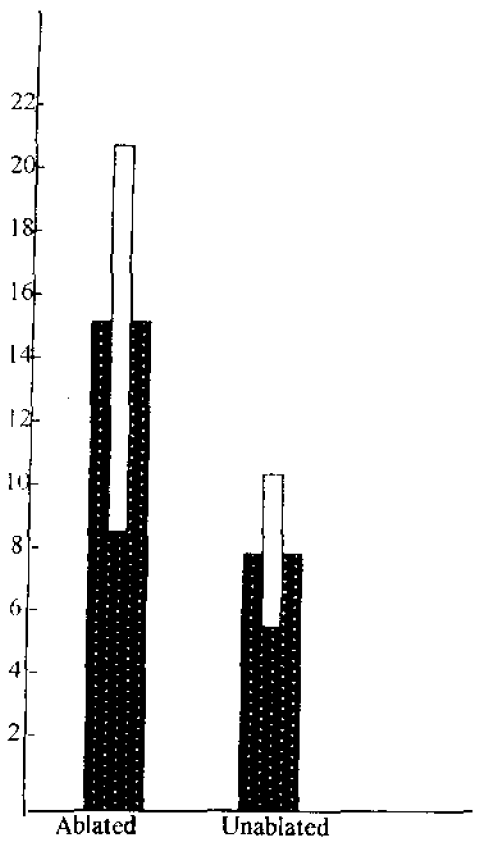


Fig no:2 showing mean and standard deviation of final percentage increase in cheliped length between ablated and unablated prawn, *M. idella* used in the experiment to study the effect of eyestalk ablation on androgenic gland and primary and secondary sex characters.



The percentage gain in body length of unilaterally ablated prawn show significant variation between ablated and unablated treatment. The percentage gain in length ranged from 10.6203 to

19 . 3697 with an average of 13 . 7819 ± 2 . 317 in ablated ones where as in unablated treatment these values were lower ranging from 0 to 13 . 8125 with an average of 9 . 1621 ± 2 . 7972 within a period of 45 days .

The data on cheliped development of ablated prawns over control given in the table shows that there is significant difference . The percentage increase in second cheliped length ranged from 6. 0203 to 26 . 5650 with an average of 14 .4115 ± 6. 031 in ablated males and from 0 to 14 . 4182 with an average of 7 . 8464 ± 2 . 8102 in ablated ones .

The data regarding other sex characters such as appendix masculina, number of spines on it. length of androgenic gland, its biomass were collected after dissecting the prawn showed that there is significant variation between ablated and unablated prawns (as seen in the Table no:8)

Table 8

t (1)	* 3.11620
t (2)	* 4 . 6166
t (3)	* 8 . 2149
t (4)	* 4 . 2538
t (5)	* 3 . 91137

* significant at 5% level

The parmeters such as length of appendix masculina t (1) , and the number of spines borne by it t (2) , the length of androgenic gland t (3) and its biomass t (4) , the width of vas deferens t (5) between the ablated and unablted prawns were found to be significant.

Table 9 Data on correlation analysis of body size and sex characters and androgenic gland of ablated and nonablated *M.idella*

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14
X1	1	**.9128	**7178	-.1818	**.7049	-.4163	*.6826	**5907	.2226	-.3015	.5097	.1082	**.8125	-.1506
X2		1	**.7238	-.1232	**.7117	-.4534	*.6738	**.7113	.1564	-.3152	*.6568	.1499	**.7666	.0809
X3			1	-.0702	**.7795	-.0945	**.8331	**.7384	.4320	-.0351	*.6891	.0314	**.8843	.0758
X4				1	-.0106	.1494	-.4575	-.1893	-.1555	**.7023	.1889	.5381	-.1425	.5553
X5					1	-.1230	**.8282	.4915	*.5703	-.3045	*6431	-.2216	**8253	.0144
X6						1	-.0635	-.4558	-.1259	.1910	.0640	.2929	-.2534	-.0476
X7							1	*.5995	.5043	-.4290	.4791	-.3373	**.7635	-.2051
X8								1	.1725	-.2804	.4169	.0011	*.6681	.2531
X9									1	-.2606	.2294	**-.5937	.3041	-.0716
X10										1	-.0387	.4254	-.1440	.2403
X11											1	.3191	*6455	.3050
X12												1	.0304	.4221
X13													1	.0861
X14														1

X1 - Length of prawn (ablated)

X2 - Length of prawn (unablated)

X3 - Length of cheliped (ablated)

X4 - Length of cheliped (unablated)

X5 - Length of appendix masculina (ablated)

X6 - Length of appendix masculina (unablated)

X7 - No : of spines on appendix masculina (ablated)

X8 - No : of spines on appendix masculina (unablated)

X9 - Length of androgenic gland (ablated)

X10 - Length of androgenic gland (unablated)

X11 - Biomass of androgenic gland (ablated)

X12 - Biomass of androgenic gland (unablated)

X13 - Width of vas deferens (ablated)

X14 - Width of vas deferens (unablated)

Analysing the correlation matrix it could be seen that some of correlation values are significant (indicated by one asteric , $p \leq .05$) and some are highly significant (indicated by two asterics , $p \leq .01$). It was found that ablation has brought in a significant variation in the second cheliped . Length of androgenic gland of the ablated prawns had shown a highly significant development. This means that destalking has resulted in a hypertrophy of the androgenic gland which in turn induces an increase in the size of the secondary sexual characters such as second cheliped , appendix masculina and also the gonad indicated by the increase in the width of vas deferens . Development of secondary sexual characters is very much influenced by the androgenic gland . This increase in the size of the androgenic gland and the corresponding sexual characters owing to the unilateral eyestalk ablation indicate that there is some hormone produced by the eyestalk having an inhibitory / restraining action on the androgenic gland, male primary and secondary sexual characters as well as on the growth of the prawn , which when removed partially by destalking brings about an acceleration in the development of these characters (table no:9).

4.3 . Observation on various water quality parameters .

TABLE 10. Range of water temperature in the experimental tanks during the study period

Temp	WEEKS 1	2	3	4	5	6	7
Mean	27 . 27	27 . 19	28 . 63	29 . 50	28 . 82	28 . 42	28 . 50
± SE	0 . 29	0 . 31	0 . 54	0 . 51	0 . 56	0 . 01	0 . 47
Range	26.98 - 27.53	26.46-27.68	27.96-29.09	28.87-30.44	27.09-29.13	28.03-28.72	27.66-29.00

TABLE 11 Fluctuations in PH values observed in experimental tanks.

Ph	WEEKS 1	2	3	4	5	6	7
Mean	7 . 52	7 . 94	8 . 06	8 . 08	7 . 88	7 . 86	8 . 06
± SE	0 . 24	0 . 31	0 . 21	0 . 41	0 . 22	0 . 19	0 . 21
Range	7 . 2 - 7 . 8	7 . 6 - 8 . 3	7 . 8 - 8 . 3	7 . 9 - 8 . 2	7 . 8 - 8 . 2	7 . 6 - 8 . 2	7 . 8 - 8 . 3

Table 12. Variations in DO_2 content in the experimental tanks.

DO_2	WEEKS						
	1	2	3	4	5	6	7
Mean	7.01	6.77	7.28	7.28	8.02	7.83	6.77
± SE	0.56	0.68	0.42	0.48	0.73	0.93	0.68
Range	6.67 - 7.35	6.8 - 7.05	7.03 - 7.8	7.16 - 7.62	7.6 - 8.32	7.35 - 8.32	6.8 - 7.0

The water quality parameters such as temperature, pH and DO_2 noted during the study period are given in the tables no: 11, 12 and 13. It could be seen that all the parameters were more or less within the optimum levels needed for the prawn, *M. idella*.

5 . DISCUSSION

5.1 Study to find the relationship between the development of sex characters with that of androgenic gland

From the study carried out to find the relationship between sex characters and the androgenic gland , it could be seen that there is a direct relationship between the sex characters and the androgenic gland , and no significant relation exists between these charecters and the body size . Individuals of similar size have varried cheliped lengths , appendix masculina and number of spines in it indicating that these characters do develop indepedent of body size .In the breeding season majority of males show different levels of hypertrophy of sex character s like the second cheliped ,the appendix masculina ,and the vas deferns . The hypertrophy of these structures is more linked with the extend of development of the androgenic gland as suggested by Thampy and John (1973) . In the present study , a positive relationship is observed between the length of second cheliped and biomass of androgenic gland . Androgenic gland is known to secrete a hormone , which determines the primary and secondary sexual characters (Thampy and John , 1972 , 1973 ; Nagamine et al : 1980) and control behavioural sexual characters according to Charniaux - cotton (1960 ; 1961) . When there is an increase in the biomass of androgenic gland , there is a corresponding increase in the length of second cheliped as found in the result obtained in the present study. It has been noted by Thampy and John (1973) that in such individuals androgenic gland shows signs of increased secretory activity as evidenced by an increase in the size of the cells, vacuolisation of cytoplasm and presence of degenerative areas . In the present study , although histological study of the androgenic gland has not been conducted it could be presumed that there is increased activity in the gland indicated by the increase in the biomass of androgenic gland . Androgenic gland being a holocrine gland (Hoffman, 1968; Thampy and John , 1972; 1973) can become active only by an increase in the biomass. The results show that with the increase in the biomass of the androgenic gland there is increase in the length of appendix masculina also . In other words it can be said that these sexual characters such as length of appendix masculina , length of second cheliped , width of vas deferens are very much influenced by the development of androgenic gland .

Different male morphotypes are reported to be available among *Palaemonids* . Thus Henderson and Mathai (1910) had noted dimorphism in the second chelipeds of males in 3 species of *Palaemon* , viz , *P . malcomsonii* , *P . dubius* and *P . scabriculum* . In *M . rosenbergii* where different male morphotypes have been noticed by Sagi *et al* (1986) each type is said to represent a different reproductive strategy : small males and blue clawed males actively taking part in mating , investing little energy on somatic growth , while orange clawed males are characterized by fast growth rate

(Sagi , 1984 ; Ra'anan and Sagi ,1985) . In the same species New and Singholka (1982) have categorised the males as bull males and feminized males . In the case of *M . idae* , Henderson and Mathai (1910) have suggested the existence of two categories of males , the ordinary and feminized males . Perschbacher et al . (1989) described feminized and normal males in *M . malcolmsonii* . In the case of *M . idae* wherein Thampy and John (1972) had observed dimorphism among males , it was found that , the length of second chelipeds , and of appendix masculina and the number of spines in it and width of vas deferens , have no relationship with the body size , these varying among individuals of the same size, well correlated with the variartion in the development of the androgenic gland . In the present study, on the basis of relationship between body size and cheliped length , it could be seen ,that a high ratio of the bodylength to cheliped length is shown by such individuals who take part actively in the breeding activity . In such individuals the ratio of body size to the androgenic gland and body size to length of appendix masculina were also found to be high . So we can assume that theses individuals take an active part in the breeding activity . In such a case these males can be considered to be ordinary males as suggested by Henderson and Mathai (1910) . Individuals whose ratio of body length to cheliped length is low can be categorised as feminized males . In such individuals , the corresponding ratio of body length to biomass of androgenic gland and of body length to length of appendix masculina were also found to be lower . So, as suggested by Henderson and Mathai (1910), in the case of *M . idella* also there are two types of males namely ordinary males and feminized males . Growth rate of males is highly variable according to Fujimura and Okamoto(1972), Smith et al.,(1978) , Brody et al., (1980) , Ra'anan (1982), Malecha et al., (1984) . Jayachandran and Joseph (1988) while studying the growth pattern of *M . idella* and *M . scabriculum* , found that the males of *M . scabriculum* exhibit isometric growth , but the females of *M . scabriculum* and both sexes of *M . idella* do not exhibit isometric growth . According to Jayachadran , (1984), the breeding season of *M.idella* extends from Aug - Dec / Jan . The present study was carried during the month of July / Aug . As such there is a possibility that these feminized males , whose ratio of various sexual characters to the body length is low, may grow to normal males, as they approach the end of the breeding season and take active part in the breeding activity .

It may be possible that androgenic gland which controls the primary and secondary sex character of male crustaceans has a role in the developement of male morphotypes and the growth variation among them , as growth rate of prawns is closely associated with morphotypic status as suggested by Ra'anan (1982)and Ra'anan and Cohen (1985) .

5.2 Effect of eyestalk ablation on the development of androgenic gland and on primary and secondary sex characters

In the experiment conducted to find out the effect of unilateral destalking on the androgenic gland and consequently on development of second cheliped ,appendix masculina and the vas deferens, it was observed that these characters significantly differ between ablated and unablated treatments . Androgenic gland which is known to secrete a hormone determines the primary and secondary sexual characters and control behavioural sexual characters according to Charniaux - cotton (1960 ; 1961) . Sex and reproduction are under the direct control of androgenic gland which in turn is under the control of x - organ sinus gland complex (Adiyodi and Adiyodi,1970). The increase in the size of the androgenic gland and the primary and secondary sexual characters by way of unilateral eyestalk ablation is indicative of the fact that there is a hormone produced in the eyestalk which has got an inhibitory effect on the androgenic gland and male primary and secondary sexual characters, as well as on the growth of the prawn . The androgenic gland being a holocrine gland enlarges as and when there is an increase in the sexual activity as observed by Thampy and John (1973) in *M . idae* . These authors also noted that when there is an increase in the length of cheliped , and appendix masculina and number of spines on it and width of vas deferens , there is a corresponding increase in the size of the androgenic gland . In other words hypertrophy of the sex charecters is the result of a hypertrophy of the androgenic gland .

Hypertrophy of the androgenic gland after eyestalk ablation was reported in several crustaceans (Adiyodi and Adiyodi , 1970) . Hyper activity and hypertrophy of glandular tissue and precocious spermatogenesis in destalked crabs suggest that androgenic gland is under the direct neurohormonal inhibitory control of x - organ sinus gland system of the eyestalk (Demeusy and Veillet , 1958) . The neuro endocrine complex produces an inhibitory hormone . When this is removed precocious gonadal development ensues. This was first confirmed in female shrimp, P.serattus and later in many decapod crustaceans such as *Cambarus* (Stephens, 1952),*Uca pugilator* (Brown and Johnes 1949), *Lysmata seticaudata*(Carlisle 1953), *Carcinus meanas*(Demeusy and Veillet 1952). Eyestalk ablation thus removes an inhibitor which is preventing gonadal maturation, thus leading to rapid uninhibited proliferation of gonadal tissue which may increase several folds.

In *P .monodon* and *P .merguiensis*, Alikunhi et al . (1975) found ablation to induce precocious gonadal development in immature male . Ablation was found to increase gonadal size in *P . setiferus* by Lawrence et al., (1979) and increased gonadal size and mating frequency in *P . vennamei* by Chamberlain and Lawrence (1981) . In the present experiment also there is an increase in the gonadal size as indicated by the increase in the width of vas deferens by destalking the

prawn *M .idella* . Leung - Trujillio and Lawrence (1985) noted that unilateral eyestalk ablated *P . vennamei* produced larger spermatophore with a more than two fold increase in sperm count over unablated controls with no effect on sperm quality .However species specific physiological , nutritional and / or environmental requirments may be involved in male reproduction as suggested by Leung - Trujillo and Lawrence (1991) which was not investigated in the present study .

Based on the experiment with bilateral eyestalk ablation and eyestalk extract injection , Kulkarni et al . (1984) concluded that hormones released from the neuro endocrine system regulate androgenic gland activity . Sarojini et al . (1994) presented the hypothesis that in the crayfish , *P . clarkii* , serotonin stimulates the release of GSH , which in turn acts upon the androgenic gland which releases the androgenic hormone . Eyestalk neuropeptides such as GSH and GIH apparently act directly on the female ovaries (Charniaux - cotton and Payen ,1988 ; Quackenbush ,1991 ;Fingerman , 1995) , whereas in males , their action on testis appears to be indirect via a direct action on the androgenic gland as suggested by Adiyodi ,(1984), Gupta ,(1989) and Hasegawa et al.,(1993) . Hence it can be concluded that the increase in the biomass of androgenic gland and primary and secondary sexual characters by way of unilateral eyestalk ablation is indicative of the fact that there is a hormone produced in the eyestalk which has got an inhibitory effect on the androgenic gland and male primary and secondary sexual characters as well as on the growth of the prawn .

In the present study it was found that unilateral destalking brings about an increase in the growth rate and also an increased growth of secondary sex characters, the rate of development of secondary sex characters being more than that of the body growth rate. This variation in the development of secondary sex characters being more pronounced than that of the body growth rate could be due to the hypertrophy of the androgenic gland. It could be seen that the increase in the biomass of the androgenic gland in the destalked ones is more than that of the control. The correlation coefficient between the biomass of androgenic gland and cheliped length is highly significant . This means that the development of second cheliped is related to the development of androgenic gland . When there is hypertrophy of androgenic gland it is reflected in the second cheliped . Similarly the development of androgenic gland is very much reflected in the width of vas deferens , length of appendix masculina and its spinuosity . The statistical analysis of the measurement of these characters shows that the hypertrophy of these structures has only very little correlation to the size of the individual and that the above sex characters have marked variation in a population having almost same body length . The hypertrophy of these structures is more linked with extent of development of the androgenic gland. According to Thampy and John,(1973) in such an individual the androgenic gland shows signs of increased secretory activity as evidenced by increase in the size of the cells ,

vacuolisation of cytoplasm and presence of degenerative areas . It is suggested that there is a direct relation between the secretory activity of the androgenic gland and the hypertrophy of these sex characters ie , there is a " cause and effect " relation between the androgenic gland and primary and secondary sex characters.

It could be seen that destalking brings about a difference in the development of androgenic gland and the sexual characters. Hypertrophy of the androgenic gland after destalking as reported by several authors in a number of crustaceans is also found in case of *M.idella*. This increase in the size of the androgenic gland and the primary and secondary sex characters by way of unilateral eyestalk ablation is indicative of the fact that there is a hormone produced in the eyestalk, which has got an inhibitory effect on the androgenic gland and on male primary and secondary sex characters as well as on the growth of the prawn .

5.3. Water quality parameters.

Different water quality parameters such as temperature, pH and DO_2 were maintained within the optimum range required for growth of *M.idella* during the experimental period.

The experiments were conducted in fresh water. Various investigators such as Wickens (1972), Venugopalan (1988) and Venugopalan and Thampy (1992) have suggested that salinity range of 0-2 ppt as ideal for growth of *M.rosenbergii*. Ignatius (1989) reported that a salinity range of 0-6ppt is ideal for growth of *M.idella*.

The temperature of water in the tanks varied between 27 and 29 0c which is reported by many workers such as Uno et al (1975), New and Singholka (1982), Sandifer and Smith (1985) as within the optimum range for growth of *M.rosenbergii*.

The pH of water in the experimental tanks varied between 7.2 and 8.3. New and Singholka (1982) and Sandifer and Smith (1985) reported a pH range of 7.5 - 8.5 as optimum for culture of *Macrobrachium* spp

The Do2 levels in the water varied between 6.6 - 8.3 ppm which was also within the optimum range required for *Macrobrachium* spp culture as reported by Subramanyam (1987). New and Singholka (1982) reported that an oxygen concentration of 75% saturation as optimum for the growth of *Macrobrachium* spp.

6.SUMMARY

The present study in the prawn *Macrobrachium idella* was taken up to find out morphotypic variations among the males and the endocrine basis for these variations and also the role if any , the eyestalk endocrines have , on the control of androgenic gland , which in turn is controlling the development of primary and secondary male sex characters .

2 . The relationship between the body size and primary and secondary sex characters and also on the androgenic gland was studied by taking a random sample of 30 males collected during July - Aug 1997. The development of secondary sexual characters such as second cheliped, and length and spinuosity of appendix masculina and the primary sex character as indicated by the width of vas deferens, have no relationship with the body size of the prawn and are well correlated with the development of the androgenic gland , which is the endocrine gland responsible for the control of male primary and secondary sex characters in the prawn .

3 . 30 males collected during the early part of the breeding season ie , July - Aug period of 1997 were grouped into two categories:

Group A - consisting of 22 individuals having a body length to cheliped ratio below 1 : 1 . 5 , have their androgenic gland not developed and the appendix masculina also not fully developed indicating a direct relationship between the development of the gland to the extend of development of sex characters . Group B - consisting of 8 individuals having a body length to cheliped ratio above 1 : 1 . 5 , have well developed androgenic gland , showing that there is a positive correlation between the biomass of androgenic gland and the cheliped length.

4 . The experiment conducted to find the effect of unilateral eyestalk ablation on growth and development of androgenic gland and of male primary and secondary sexual character, for a period of 45 days, had shown that destalking brings about a positive change in the development of androgenic gland and sex characters .

5 The percentage gain in body length of unilateraly ablated treatment shows significant variation between ablated and unablated treatments. The percentage gain in body length ranged from 10 . 6203 to 19 . 3697 with an average of 13 . 7819 $\pm$ 2 . 317 in ablated ones whereas in unablated treatment these values were lower, ranging from 0 . to 13. 8125, with an average of 9 . 1621 $\pm$ 2 . 7972 within a period of 45 days .

6. Development of cheliped of ablated prawns over control can be seen to be quite significant. The percentage increase in second cheliped length ranged from 6.0203 to 26.5650 with average of 14.4115 ± 6.0315 in ablated males, while it is only 0 to 14.4182 with an average of 7.8464 ± 2.8102 in unablated ones.

7. The other parameters such as length of appendix masculina, number of spines on it, biomass of androgenic gland, width of vas deferens etc. between the ablated and unablated prawns were found to be significant. the differences being contributed by the enlargement of the androgenic gland owing to the removal of inhibition over it by eyestalk endocrines,through destalking.

8. Analysing the correlation matrix, it could be seen that eyestalk ablation has brought in a significant variation in body length as well as in the length of second cheliped.

9. Biomass of androgenic gland of the ablated prawns shows a high degree of development. This means, by destalking, there is a hypertrophy of the androgenic gland which in turn is manifested by increase in the secondary sexual characters such as second cheliped, appendix masculina and gonad as is indicated by the increase in the width of the vas deferens.

10. The increase in the size of the androgenic gland and the primary and secondary sexual characters by way of unilateral eyestalk ablation is indicative of the fact that the GIH, a hormone produced in the eyestalk has got an inhibitory effect on androgenic gland and male primary and secondary sexual character as well as on the growth of the prawn.

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EFFECT OF EYESTALK ABLATION ON ANDROGENIC GLAND AND MALE SECONDARY SEXUAL CHARACTERS IN *MACROBRACHIUM IDELLA* (Hilgendorf)

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ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement for the degree

MASTER OF FISHERIES SCIENCE

FACULTY OF FISHERIES

KERALA AGRICULTURAL UNIVERSITY

DEPARTMENT OF AQUACULTURE

COLLEGE OF FISHERIES

PANANGAD , COCHIN

1998

ABSTRACT

The present study in the prawn, Macrobrachium idella was taken up to find out morphotypic variation among the males, the endocrine basis for these variations and also to know the role if any, the eyestalk endocrines have, on the control of androgenic gland, which in turn is controlling the development of male primary and secondary sex characters.

The study conducted to find the relationship between development of sex characters with that of androgenic gland conducted using 30 male prawns of different sizes has shown that these sex characters are under the control of androgenic gland and have no relationship with that of the body size. Of the 30, 22 individuals were found to have a bodylength to cheliped length ratio below 1:1.5, their androgenic gland and appendix masculina, not fully developed indicating that they are reproductively inactive. Remaining 8 individuals have a bodylength to cheliped length ratio above 1:1.5. They have well developed androgenic gland and appendix masculina showing that there is positive correlation between the biomass of androgenic gland and the cheliped length.

An experiment conducted to find out the effect of unilateral eyestalk ablation on growth and development of primary and seconadry sexual characters as well as on the androgenic gland, had shown that destalking brings about a positive change in the development of androgenic gland and sex characters.

The increase in the size of the androgenic gland and consequent hypertrophy of primary and secondary sexual characters brought about as a result of unilateral eyestalk ablation do indicate that the GIH, a hormone produced in the eyestalk has got an inhibitory effect on androgenic gland as well as on the growth of the prawn, which when removed by " destalking" , results in the enlargement of the androgenic gland and other sexual characters.