EFFECT OF EYESTALK ABLATION ON GROWTH AND REPRODUCTION OF *MACROBRACHIUM EQUIDENS* (Dana)

By
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THESIS

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TO
MY
PARENTS
AND
SISTER

DECLARATION

I hereby declare that this thesis entitled EFFECT OF EYESTALK ABLATION ON GROWTH AND REPRODUCTION OF MACROBRACHIUM EQUIDENS (Dana) is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree diploma associateship fellowship or other similar title of any other University or Society

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I INTRODUCTION

The rapidly growing prawn aquaculture industry involving the culture of commercially important decapods calls for crustacean endocrinology to assist in devoleping techniques to increase crop yield by stimulating growth and reproduction (Fingerman 1987) The control of reproductive cycle of an animal of commercial interest is an important prerequisite for programming its culture 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 the control of gonadal devolepment in crustaceans the technique of eyestalk ablation is receiving greater attention as a method of inducing precocious maturation in captivity (Choy 1987) It seems that it may retain its importance in the foreseeable future

Eyestalk ablation capacitates to treat the animals for inducing maturation individually with great simplicity Ablation leads to predictable peaks in maturation and spawning which facilitates the setting up of production schedules in contrast to scattered spawns for unablated females For production purposes this predictability in breeding success and the increase in number of broods per individual can adequately compensate towards decreased fecundity if any

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 culture of freshwater prawn Macrobrachium spp is a recent devolepment The giant freshwater prawn M rosenbergii is now being cultured in different parts of the world in countries such as Thailand Malaysia Taiwan Vietnam USA, Brazil Mexico Mauritius French Guiana French Polynesia Dominican republic and Japan The production has reached 22995mt which constitute 35 percent of freshwater crustaceans and 0 08 percent of the total aquaculture production (FAO, 1992) Commercial production of the seed of M rosenbergii was initiated by the pioneering work of Ling (1977) which evoked widespread interest for taking up commercial culture of M rosenbergii and other species The major constraint for devolepment of commercial farming of freshwater prawn is still the non availability of seed in most of the countries Recent devolepments in the hatchery production of M rosenbergii has increased the prospects of culture of this species considerably However the devolepment of freshwater prawn culture will largely depend on utilization of other larger species such as M malcolmsonii M acanthurus and M tellinium and many medium sized species such as M idella M rude M villosimanus and M equidens In this context species like Macrobrachium equidens though medium sized can be considered as a potential candidate (Natarajan et al 1979 Ignatius 1989 Ignatius and Thampy 1991) M equidens has attractive attributes towards aquaculture in that it is tolerant to a wide range of salinity and is hardy

The production of seed under controlled conditions is vital for the success of any aquaculture venture Any attempt in seed production under controlled condition calls for a full understanding of the mechanism involved in the control of reproduction Eyestalk ablation as a method of inducing ovarian devolepment and ovulation is used in freshwater prawns also Enhanced growth has also been reported in eyestalk ablated prawns like M malcolmsonii (Murugadass et al 1988) In view of these the present study was taken up to find the effect of eyestalk ablation on growth and reproduction of both females and males of a medium sized cultivable palaeomonid prawn M equidens available in the brackish water areas along the southwest coast of India

II REVIEW OF LITERATURE

2 1 Distribution of Freshwater Prawns

Species of the freshwater prawns of the genus Macrobrachium are distributed throughout the tropical and subtropical regions and about 100 species are known to exist today Of these many are cultivable and used for inland aquaculture The largest and most popular among them the giant freshwater prawn Macrobrachium rosenbergii is indigenous to South East Asia Northern oceanica and Western Pacific Islands (New and Singolka 1982)

In India the genus is represented by about 40 species out of which 15 are important from fisheries point of view (Jayachandran and Joseph 1992) M rosenbergii which is distributed from Indus delta of India China to Asian mainland in fresh and brackish water areas is available in West Bengal Gujarat and in Kerala M malcolmsonii the species next in importance which is distributed in India is found in the peninsular rivers that drain into the Bay of Bengal M choprai which completes its life cycle in freshwater is distributed in Allahabad Varanasi Buxar Patna Bhagalpur and Lagola centres in the river Ganges (Tripathi 1992)

M *idella* having a distribution in Indo west pacific East Africa Maaagascar and India, *M. scabriculam* distributed in Indo west pacific east Africa and Madagaskar to India Srilanka Bangladesh and Sumatra and *M. equidens* distributed in Indo west pacific Nigeria and Madagaskar to south china New Britan and New calidonia are the three species of medium sized *Macrobrachium* which are available in sizable quantities in the Kerala backwaters and canals Of these the first (*M. idella*) one supports a significant fishery in many parts of Kerala especially in Vembanad lake where Kurup *et al.* (1992) have reported an annual catch of the order of 68 3 tonnes (58%) , *M. scabriculum* is reported to contribute 6 78 tonnes (7 6%)

M *equidens* found mostly in brackishwater areas is distributed in Kerala only north of Cochin backwaters and it formed 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 Jayachandran and Joseph (1989 described the spotted and striped varieties which exist in these species as two sub species namely M *equidens equidens* and M *equidens pillay* respectively Recently Pillay (1990b) has given the striped form the status of a new species _M striatus_ In a recent study Sebastian (1993) has established the identity of these two species bringing in support data from electrophoresis and oogenisis Although it is only a medium sized prawn _M equidens_ has a potential for culture especially in brackish water areas (Natarajan _et al_, 1979 Ignatius 1989 Ignatius and Thampy 1991)

2 2 Growth

Growth rate is considered as a trait of great economic value for all species used in aquaculture Growth in prawns is a discontinuous process interspersed with the process of moulting

2 2 1 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_ (Gydamo 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 significantly affected by eyestalk ablation in the species such as _P_ monodon (Emmerson 1983) and Paralithodes camschatica (Moly neaux and Sheirley 1988)

Koch (1952) working on the crab Eriochier sinensis 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 americanus The difference between ablated and unablated they could observe was the lower value of lipid deposition in the muscle and hepatopancreas and an accelerated growth in protein in the case of ablated specimens Murugadass et al (1988) working on the freshwater prawn Macrobrachium malcolmsonii reported accelerated growth for destalked specimens of both sexes which they have attributed to the less energy being channelised towards exuvial production Accelerated growth rate achieved as a result of increased moult frequency with increased weight gain was reported in the case of Penaeus canaliculatus (Choy 1987) Carlisle (1955) while attributing faster moulting rate to be due to the removal of MIH factor from the eyestalk suggested the elimination of the hormone that regulated water uptake during ecdysis as the factor responsible for the higher percentage of weight gain as eyestalkless lobsters become abnormally large after several moults

2 3 Reproductive biology of *Macrobrachium* spp

2 2 1 Sexuality

Palaemonid prawns are dioecious the sexes being distinguished by a number of external characters (Patwardhan 1937) In general the females are smaller than the males of the 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 musculina in the endopodite of second pleopod In females 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 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 on a raised papilla on the inner side of the coxa of the third walking legs

2 3 2 Sex ratio

Seasonal variations in sex ratios have 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 Ø 17 to 1 5 87 in the case of M rosenbergii the females dominating during September to December and males during March to June and 1 Ø 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 palaemonids reach sexual maturity within a year as reported for M rosenbergii (Raman 1967) Goorah and Parameswaran (1983) recorded 118 mm and 2Ø gm as the smallest size at maturity of this species The size at maturity of M malcolmsonii has been reported as 41 mm (Ibrahim 1962) and 4Ø 5Ø mm (Sankolli and Shenoy 198Ø) Jayachandran (1984) reported the age of maturity of M idella as 12Ø days Pillay and Mohammed (1973) also reported it as 12Ø days under laboratory conditions Pillay (199Øa) reported that M equidens had reached maturity at a size of 41 48mm in captivity

2 3 4 Breeding season

The breeding season of freshwater 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 the breeding season of M equidens as from August to January/February while Pillay (1990a) noted it as August to November

2 3 5 Male morphotypes

Different male morphotypes are reported to be available among Palaemonids Thus Henderson and Mathai (1910) noticed dimorphism in the second chelipeds of males in three species of Palaemons such as Palaemon (Macrobrachium) *malcolmsonii* *P* *dubius* and *P.(Macrobrachium)* *scabriculum* In *M* *rosenbergii* where different male morphotypes have been noted (Sagi *et al* 1986) each type is said to represent a different reproductive strategy (Sagi *et al* 1990) small males and blue clawed males actively take 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 *Macrobrachium* *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 (1973) had observed dimorphism among males the length of second cheliped the length of the appendix masculina the number of spines in the appendix masculina and the width of the vas deferens have no relationship with the body size these varying among individuals of the same size well correlated with the variation in the development of the androgenic gland

2.3 6 Moulting and reproduction in females

In freshwater prawns ovarian maturation proceeds through intermoult and culminates in prespawning moult (Narayanan and Adiyodi 1992) This prespawning moult is of great significance among palaemonids in which new breeding dress is acquired by females in order to carry eggs Mating takes place a few hours after this moult According to Joshi and Diwan (1992) the ideal time for copulation is between 2 5-4 hours after prespawning moult as evidenced by the success in artificial insemination during this period in M idella The female exoskeleton is likely to harden resulting in failure of insemination after this period Male deposits sperms on the ventral side in between the second and fourth pareiopods of females The production of pheromone is reported to be there during the time of spawning moult by the female which serves in attracting males Further Jayachandran and Jose (1993) reported the possible role of male pheromone involving in the advanced devolepment of ovary They have observed an inhibition of ovarian devolepment and ovulation in long term male deprived females of M idella

2 3 6 1 Spawning

Spawning closely follows mating, but neither mating nor presence of a mating partner is a prerequisite for oviposition in species of Macrobrachium such as M. australience (Lee and Fielder 1982) M idella (Narayanan and Adiyodi 1992) M rosen_ bergii (Wickins and Beard 1974) and M heterotrichius (Ching and Velez 1985) In *M.idella* spawning occurs 10 12 hours after moulting of the females (Narayanan and Adiyodi 1992)

In many palaemonids although oviposition may occur even without mating such eggs fall off after 2 or 3 days Brachyurans such as *Gecarcinus lateralis* (Klassen, 1975) and *Paratelphusa hydrodromous* (Krishnakumar 1985) are a step ahead in conditioning mating as a prerequisite to spawning thereby successfully avoiding the otherwise wasteful process of laying unfertilized eggs The caridean shrimp *Caridina natarajani* also fails to spawn in case there is no mating thereby avoiding the laying of unfertilized eggs (Thampy 1972)

According to Wickins and Beard (1974) in palaemonids ovarian growth and somatic growth are tuned as synergistic and specific stages in oogenisis programmed strictly in relation to specific stages of moult cycle Narayanan and Adiyodi (1992) reported that each stage of ovarian growth corresponds with a specific stage in moulting cycle in *M idella* However in peneaids ovarian growth and somatic growth are reported to be antagonistic processes

The tendency to skip an ovarian cycle during an ecdysial cycle has been reported in *M idella* (Narayanan and Adiyodi

1992) M *nobilii* (Pandian and Balasundarum 1982) and M *rosenbergii* (Wickins and Beard 1974) named as resting (neutor) moult cycle (Bomırski and Kelk 1974) This may relate to some changes in environmental condition and/or stress during handling and overcrowding in transportation

2 3 6 2 Fecundıty

In several malacostracans the relative number of eggs exhibits a linear relationship to the size of the mother (Jensen 1958) as reported in M *rosenbergii* (Costa and Wanninayake 1986 Patra 1976) M *rude* (Shakunthala 1976) and M *lanciforns* (Rasalan *et al* 1980) Those species of *Macrobrachium* which complete their life cycle in freshwater produce fewer eggs and pass through fewer larval stages The migratory species produce larger number of eggs to neutralize the risk that the larvae will have to undertake (Pandian 1987) However the total number of eggs produced per unit of body weight decreases with increase in size (Villegas *et al* 1986)

Fecundity for M *rosenbergii* was reported as 19 000-1 37 000 (for 12 31 cm size Costa and Wanninayake 1986) 54 000-2 76 000 (Patra 1976) 60 000 1 00 000 (Ling 1969) 40 000 (for 45 cm size Malecha 1983) and 1216 89 747 (for 9-15 8cm total length and 6 22 45 8g weight Ang and Kok

1991) For M malcolmsonii it was 3500 64 000 (Mathavan et al 1986). In the case of M. vollenhovenii it was 1 160 1 70 670 (for 5 45 13 5cm size-Udo and Ekpe 1991) The figures reported were 52 70 in M lammarrei (Manna and Raut, 1991) and 30 130 in M. davanum (Manna and Raut 1991)

Malecha (1983) found that the fecundity of tank reared brooder and that of the wild/pond reared brooders of M rosenbergii varied significantly

2 3 6 3 Incubation

In freshwater prawns the eggs are carried underneath the abdomen in a brood pouch cemented to among the setae in the pleopods During the spawning moult there is a drastic change brought about in the brood pouch which forms part of a breeding dress newly acquired during this period The developing eggs are ventilated by the fanning activity of the pleopods of the mother to facilitate gaseous and ionic exchange for eggs during incubation Incubation of eggs is reported to be an energy demanding process (Mathavan and Murugadass 1988) The incubation period varies with species as listed in table 1

Table 1 **Incubation period of *Macrobrachium* spp**

Species	period (days)	Source
Macrobrachium rosenbergii	15-24	Rao 1986
	16	Diaz 1987a
	17	Diaz 1987b
	19	Ling, 1969
	19-20	George 1969
M acanthurus	14	Martinez Silva 1981
	16 18	Choudhury 1971
	16-18	Carr 1979
M amazonicum	12 15	Guest 1979
	15 17	Magalhaes 1985
	15 20	Romero, 1982
M idella	14 16	Natarajan 1947
	14 8-14 54	Ignatius 1989
M kistensis	29-30	Nagabhushanam and Kulkarni 1979
M lanceifrons	22	Rasalan *et al.* 1969
M malcolmsonii	15	Kevalramani 1973
M equidens	16	Pillay 1990a

The presence of developing eggs in the brood pouch delays moulting (Schone 1961) The parental care provided by the incubation offers greater survival of the eggs (Villegas *et al* 1986)

2 3 6 4 Hatching

During hatching the mother creates a powerful water current by beating the pleopods and as a result the eggs hatch During this process the mother preens the egg mass with maxillipeds to sever the eggs The exposure to strong current and the preening of the eggs might trigger hatching (Balasundaram and Poyyamoli 1984) However when disturbed the pleopod beat frequency decreases (Balasundaram 1980) and the female postpones the process of hatching Batch hatching is controlled by mother and not by the stage of developing eggs in M nobilii (Balasundaram and Pandian 1981), but can be due to the presence of an unfavorable environment (Balasundaram and Poyyamoli 1984) In the case of M idella Ignatius (1989) has observed that the incidence of batch hatching is more at 0,12 and 18 ppt salinities compared to 6 ppt Exposure to estuarine environment however seems to be obligatory for the first moult of non feeding zoea of the estuarine species of Macrobrachium(Pandian 1987)

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 male gonopore complex and mature musculine cheliped and the initiation of spermatogenesis in the ovaries (Sagi ,1988) However spermatogenesis is not initiated in mature females (Nagmine et al 1980) Andrectomy either affects the quantity of sperm produced or the rate of production (Sagi et al 1990)

The androgenic gland being a holocrine gland enlarges as and when there is an increase in activity The increase in biomass 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 musculina and the width of vas deferens there is a corresponding increase in 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)

The differential growth shown in Macrobrachium the males growing faster than females (Sagi et al 1986) adds to the significance of this gland The growth rate among males vary greatly (Fujimura and Okamoto 1972 Smith et al 1978 Melecha et al 1984) resulting in the existence of different morphotypes (Ra anan and Cohen 1985 Kuris et al 1987) In all forms 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 the precocious spermatogenisis in destalked crabs suggested that the androgenic gland is under the direct neurohaemal inhibitory control of the X organ sinus gland system in the eyestalk (Demeusy and Veillet 1958)

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 overiactomy 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 the 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 nataragini (Thampy 1972).In the case of C nataragini Thampy (1972) noted that females which had undergone spawning moult but not provided with a male resorbe the yolk by the 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 the breeding dress 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 devolepment / retention of the breeding dress

The overaiactomy 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 prove that the permanent and temporary secondary sexual characters in female crustaceans are under the control of ovarian hormone/s

2 4 2 Endocrine control of reproduction

Reproduction in Crustacea like many physiological processes is under endocrine control Moulting and reproduction are major metabolic events involving cyclic mobilization of organic reserves from storage depots to the epidermis and gonad respectively and though temperolly separated are functions inseparably integrated with one another (Adiyodi and Adiyodi 1970)

The eyes in decapod crustaceans are generally stalked and movable and are known to contain a variety of hormones or factors apparently responsible for growth moulting metabolic rate water balance dispersion of retinal pigments and sexual activity (Lockwood 1968) The active principles appear to be elaborated by groups of neuroscretory cells located in areas such as optic lobe peduncle contained within the eyestalk the brain and thoracic ganglion The neurohormones usually are transported intra axonally through well defined pathways and stored in the axon terminals until under the appropriate stimulus they are released to the blood The presence of a hormonal principle was first brought to light by the pioneering experiments of Panouse (1943) The X-organ sinus gland complex is believed to produce two hormones the one controlling reproduction (Ovary Inhibiting Hormone) and the other the process of moulting (Moult Inhibiting Hormone) (Adiyodi and Adiyodi 1970) The medulla externa is considered to play a principal part in inhibitory mechanism of the ovary (Nakamura 1985) The ovary inhibiting hormone was found to be effective in males also and so the name gonad inhibiting hormone was suggested for it (Adiyodi and Subramoniam 1983) Adiyodi (1980) noticed that though a high titre of GIH inhibited gonadal growth a low level of GIH is needed for the maintenance of gonadal growth and so suggested the name gonad restraining hormone Eyestalk ablation extirpation or more specifically destruction of the pars ganglionaris x organ or its homologue leads to precocious moulting and reproduction (Adiyodi and Adiyodi 1970)

In the case of male crustaceans destalking of young or nonbreeding adults is known to induce enlargement of vasa deferentia (Demeusy 1953 1967a b Otsu 1961 63 Gomez 1965) and hypertrophy and hypersecretion of androgenic gland Physiological (Bliss 1960) and histological (Adiyodi 1967) evidence suggest a low titre of GIH during intermoult in reproducing individuals.

Mohammed and Diwan (1991) working on P indicus observed that the neurosecretory cells of the vacoular and secretory phase dominated the MTGXO and MEGXO in the eyestalk of immature females Subsequently in the ripe stage quiescent stage neruosecretory cells dominated in these centres The sinus gland was swollen with disulphide rich secretory granules in the immature stage and it was devoid of any neurosecretory material during the mature stage (Mohammed and Diwan 1991)

A high titre of Moult Inhibiting Hormone (MIH) is considered essential for reproduction which helps the reproductive process indirectly by restraining the Y organ (Adiyodi and Adiyodi 1970) According to Adiyodi and Adiyodi (1970) gonadal development heralds a shift from the state of synergism between MIH and GIH such as to one of antagonism in the intermoult the shift necessitated by the demands of the gonad particularly on metabolic reserves, in such a way as to interface temporally the growth of the integument It is possible that the X organ can produce GIH as well as MIH which are chemically related molecules (Adiyodi and Adiyodi 1970) In M.nipponense Han and Kim (1993) observed by resorting to eyestalk ablation that the effect of X-organ which inhibit the gonadal maturation (GIH) was stronger in resting period than in previtellogenic and early spawning period However when vitellogenesis is already in full swing eyestalk ablation does not perceptibly accelerate ovarian growth suggesting that during this period the synthesis of GIH and/or its release into general circulation may be very low (Adiyodi and Adiyodi 1970 Kulkarni and Nagabhushanam 1980 Han and Kim 1993) The observations of Stephens (1952), Drach (1955) Hoffman (1968) and Cheung (1969) had indicated that gonad maturation and therefore the GIH secretion like MIH secretion (Passano 1960 Aiken 1969) may be under the direct influence of such factors as the season photoperiod thermoperiod, nutrition salinity and other stimuli acting through sensory receptors

A gonad stimulating hormone/principle (GSH) is known to be produced by the brain (Gomez and Nayar 1965 Yano and Wyban, 1992) and thoracic ganglion (Rao et al 1981) In P vannamei Yano and Wyban (1992) observed that ovarian maturation is induced by a brain hormone which stimulates the release of gonad stimulating hormone from thoracic ganglion The role of GSH is dual that is to promote oocyte growth and to prevent the Y organ activity (Adiyodi and Adiyodi 1970) In males the implantation of brain and thoracic ganglion results in the precocious maturation of the testes and eventually the hypertrophy of the vasa deferntia (Gomez 1965)

GIH GSH and MIH in Crustacea are neurohormones whereas moulting hormone is epithelial in origin and is a steroid (Adiyodi 1980) The presumed moulting hormone of crustaceans was isolated purified and its structure determined by Hamshire and Horn (1966) who renamed it as ecdysone 20 β hydroxy ecdysterone

Anilkumar and Adiyodi (1985) suggested the existence of eyestalk hormones controlling oviposition and stated that they are released at least one week prior to spawning in the crab Paratelphusa hydrodromus

2 5 Methods of inducing maturation in prawns

Prawns are induced to mature using three different techniques which can be catogerised as endocrine environmental and nutritional

2 5 1 Environmental

All animals are known to select the most appropriate time for breeding As such the fluctuations of environmental factors have a definite influence in stimulating maturation and subsequent reproductive processes The important among the environmental factors are light salinity, temperature dissolved oxygen pH ammonia and nitrite In maturation systems these factors are set at optimum levels

2 5 1 1 Light

The presence of an eyestalk mediated control mechanism for reproduction in shrimps emphasizes the importance of light on maturation Increase in photoperoid resulted in gonadal maturation of P japonicus (Laubier and Laubier 1979) and P kerathurus (Lumure 1979) in the subtropical regions where there is a profound variation in photoperiod with season However it is unlikely that photoperiod has any profound effect on tropical prawns accustomed to a more or less equal day/night regime throughout the year

The intensity of light is known to affect the maturation In nature, prawns breed at the bottom (Muthu 1983) where the light intensity is very low Hence strong light can act as a source of stress to the prawns especially non burrowing ones (Brown et al 1979) evidenced by the delayed maturation in outdoor ponds or cages

Although moulting in captivity has not been observed to be synchronized a greater number of females moulting in the light phase of the moon is in agreement with that observed for penaeid prawns in wild (Kirkegard and Walker 1970) Black colour of the tank is comparatively more suitable than other colours because it absorbs more light enabling the female to mature more properly (Emmerson 1980)

2 5 1 2 Temperature

Temperature of water in the maturation facility is bound to influence the rate of all physiological functions including the rate of maturation Controlling temperature has been instrumental in inducing maturation in temperate prawns such as P japonicus (Laubier 1978) and P esculentus (Crocos and Kerr

1986) However in tropics this technique is less effective

2 5 1 3 Salinity

Salinity as a factor has a profound influence on gonadal development For normal development of gonad the optimum levels of salinity is to be maintained Studies on the influence of salinity on reproduction of palaemonids are very limited Salinity is known as a major factor limiting the reproductive capability of those Macrobrachium which undertake breeding migration In the case of M idella Ignatius (1989) observed that a salinity level of 6 12 ppt is congenial for ovarian development as compared to a lower level of Ø ppt and a higher level of 18 ppt In many species as in Macrobrachium rosenbergii the early growth phase is spent in the upper streches of the rivers and the adults migrate to lower reaches for breeding The number and size of egg in M nipponense in freshwater is found to be different from those living in brackishwater region (Moshiko 1983 1984)

2 5 1 4 pH

pH as a factor is known to influence the gonadal development A higher pH of 8 2 induces maturation in P *indicus* (Muthu *et al* 1984) Similar higher values of pH such as 7 1 8 6 (Emmerson 1980) 7 8 8 1 (Prımavera *et al* 1982) 8 2 (Aquacop 1983) 7 1 8 6 (Emmerson *et al* 1983) for *P indicus* and 8 2 (Aquacop 1977 1979 1980 1983) 7 8 8 1 (Primavera 1978) 7 9 8 1 (Pudadera and Primavera 1981), 7 8 8 (Ruangpaint *et al* 1981) for *P monodon* have been re ported The optimum pH reported for *M rosenbergii* is 7 8 5 (New and Singholka 1982)

2 5 2 Nutritional methods

Nutritional requirements for reproduction in prawns are poorly defined (Bray and Lawrence 1990) Not much effort has been directed towards evaluating the relative suitabilities of various types of food for maturation (Nascimento *et al* 1991) Broodstock prawns are generally fed *ad libitum* wıth fresh and frozen clam (Muthu and Laxminarayana 1979) bloodworm (Lytle *et al* 1990) squid meal (Aquacop 1975 1977 1979) marine worms (Ponnuchamy *et al* 1981) shrimp (Beard *et al* 1977) fish (Halder 1978 80) mussels and oysters (Arnstein and Beard 1975 Beard *et al* 1977 Primavera *et al* 1978 Caubere *et al* 1979, Laubıer and Laubıer 1979) supplemented with dried pellets (Moore *et al* 1974 Aquacop 1977 79) Feeding with mixed ration has given most steady results in various species

Maturing ovaries which account for about 10 per cent of the total wet weight of the organism (Bray *et al*, 1989) have been shown to contain a large lipid component In *P setiferus* it is approximately 17 5 per cent of the ovarian dry weight for wild collected females and 20 per cent for captive females

Considering the fact that the ovaries require a high level of lipids and that the hepatic storage capability of lipids is limited when there is accelerated ovarian development due to eyestalk ablation there will be a higher dietary lipid requirement than that reported for non reproductive animals Lipids play a major role as cell constituents in the process of spawning embryogenesis and hatching (Teshima *et al* 1988)

The poly unsaturated fatty acids C 20 4 C 20 5 and C 22 6 are found to be essential components of ovarian lipid in peneaid prawns (Middleditch *et al* 1979) Prawns are not capable of synthesising these fatty acids (Teshima and Kanazawa 1983) and hence should be provided in adequate quantity in the diet for proper ovarian growth The balance between ω3 and ω6 fatty acids is an important entity of maturation diet (Lytle *et al* 1990)

Cod liver based diets supplemented with natural food such as squid mussel and annelids were found superior to soyabean based diet in terms of number of spawnings fecundity, egg and nauplii production and hatching rate of eggs The fatty acid profile of the cod liver oil based diet more closely resembled the fatty acid profile of the maturing ovaries (Millamena et al 1985)

Sterols in sufficient quantity are required in diets of crustaceans and the composition of sterols appears to be less critical than fatty acid, cholesterol or other sterols can be used as a source for this

Phospholipid requirement for lipid transportation which is an important step in reproduction has been suggested (Teshima and Kanazawa 1983) D Abramo et al (1981) noted the importance of phosphatydyl choline as a component of lipoprotein complex that transfer cholesterol from hepatopancreas to haemolymph Millamena et al (1986) reported that lecithin as an additional lipid source improved the fecundity of females and survival rate of male broodstock in P monodon, but produced lower hatching rates as compared to cod- liver oil Excessively high levels of 18 2 w6 and 18 3 w6 fatty acids in the diet were also reported to have toxic effect on prawns

Deshimaru and Shuigeuno (1972) found that the aminoacid profile of clam meat and squid meal is similar to that of prawns and hence these two are excellent sources of proteins for cultured prawns Similar qualities have also been attributed to other natural food such as blood worms and polychaete worms (Brown *et al* , 1979 1980 Chamberlain and Lawrence 1981)

The effect of ascorbic acid on the sperm and spermatphore 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 6 3 Endocrine methods

2 6 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 oogenisis of sand shrimp Crangon crangon (Bomirski and Kelk 1974) and vitellogenin synthesis in Idothea balthica (Souty and Picaud 1984) Similarly progesterone produced ovarian maturation in prawn Parapeneopsis hardwickii (Kulkarni and Nagabhushanam 1979)and in Metapenaeus ensis (Yano 1985) and bovine gonadotropin follicle stimulating hormone and leutinizing hormone in Crangon crangon (Zukowska Arendarezyk 1981)

2 6 3 2 Eyestalk ablation

Removal of eyestalk results in precocious maturation of ovaries (Panouse 1943) and testes (Demeusy 1953) Interbreeding and intermoulting period can be shortened by eyestalk ablation (Subramonium 1988) The knowledge of the X organ sinus gland complex secreting hormones having inhibitory roles on the reproduction and moulting process has lead to the extensive use of eyestalk ablation technique to induce maturation in prawns A number of workers tried to induce maturation in the prawns and shrimps by resorting to eyestalk ablation (vide table no 2)

With the increasing knowledge of endocrine activity and its control on gonadal development in crustaceans the technique of eyestalk ablation is receiving increased acceptance as a method of inducing precocious maturation of ovary and subsequent spawning

Table no 2 **Eyestalk ablation experiments on reproduction in shrimps and prawns**

Species	Source
Artemesia longinaris	Paterilla and Diaz 1987
Carıdına rajadharı	Persis and Sarojini 1985
Macrobrachium nobilii	Kumari and Pandian 1987
M malcolmsonii	Murugadass et al.,1988
M. idella idella	Jayachandran and Jose 1993
Palaeomon paucidens	Kamaguchi 1971
Penaeus aztecıs	Aquacop 1977 1979
P. canalıculatus	Choy 1987
P duorarum	Idyll 1971 Callıouet 1973
P indıcus	Emmerson 1980 Muthu and Laxminarayana 1979 Primavera et al. 1982 Makinouchi and Primavera 1987
P japonicus	Oltra and SanFeliu 1990
P kerathurus	Lumure 1979 Oltra and San Felıu 1990
P merguiensis	Alikunhi et al. 1975 Beard et al. 1977
P monodon	Alikunhi et. al. 1975 Chen 1977 Aquacop 1977 1979 1980 Santiago 1977 Halder 1978 Primavera 1979 Rodriquez 1979 Rajyalakshmi et al. 1988 Beard and Wickins 1980 Emmerson 1983 Pernomo and Hamami 1983 Chan 1991 Tan Fermin 1991
P orientalis	Arnstein and Beard 1975
P plebejus	Kelmec and Smith 1980
P semisulcatus	Browdy and Samocha 1985a b
P schimitti	Bueno 1990
P setiferus	Brown et al. 1979
P stylirostris	Aquacop 1979 Bray et al. 1989 Bray and Lawrence 1990
P yanammei	Aquacop 1979 Chamberlain and Lawrence 81a,b Yano and Wyban 1993

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 result in high mortality Calliouet (1973) performed ablation by cutting the eyestalk near the base with the help of sharp scissors and the wound cauterised immediately with a pencil type soldering iron to avoid loss of haemolymph Primavera (1978) incised the eyeball with a sharp blade allowed the fluid to ooze out and then squeezed out the contents of the eyeball outwards two to three times to destroy the tissue Rodriquez (1979) crushed out the eyestalk by pressing between fingers The last two methods are suited only for hard shelled species like P monodon

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 per cent survival of the destalked animals

Makinouchi and Primavera (1987) comparing different techniques of ablation in P indicus reported 90 100 per cert survival in eyestalk cauterised females which was comparable to the unablated treatment Significantly low survival rate was recorded in females which were ablated by pinching resulting from stress

Ablation has been done both unilaterally and bilaterally and the latter resulted in high initial mortality In this case though full development of gonads was observed spawning did not take place the ovaries regressed gradually and the prawns died within a month Loss of balance and spiral movement were also observed However Alikunhi et al (1975) and Rajyalakshmi et al. (1988) reported spawning in bilaterally ablated prawns The high mortality and inability to spawn after bilateral removal of eyestalk prompted researchers to abandon this method (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 relating reproduction (Emmerson 1980) Ideally ablation should be undertaken during intermoult for maturation to follow during premoult leads to moulting and subsequently longer latency period (Aquacop 1979 Primavera 1979) and during postmoult to mortality because of added stress of the female and excessive loss of haemolymph (Aquacop 1979) Animals ablated early in the moult cycle get enough time between ablation and spawning (Browdy and Samocha 1985).

The latency period from ablation to the onset of maturation depends on age source of broodstock and the stage of moult cycle(Muthu and Laxminarayana 1982) The number of moults before which maturation is induced is reduced by eyestalk ablation (Aquacop 1982 Crocos and Kerr 1986)

Browdy and Samocha (1985a) reported a significant decline in mating success in ablated P semisulcatus females Muthu and Laxminarayana (1984) conducted artificial impregnation in unilaterally ablated specimens of P indicus and reported that such a technique can yield viable egg which could be reared to post larvae and subsequent stages Eyestalk ablated specimens of P canaliculatus mated at much smaller sizes (Choy 1987)

The number of eggs spawned may vary according to species The effect of unilateral ablation on spawn size is poorly defined (Browdy and Samocha 1985a) Primavera et al. (1982) and Santiago (1977) reported no significant effect of ablation on fecundity Microscopic examination suggested the progeny of unilateral ablation to be normal and viable (Santiago 1977) Increment in fecundity has been demonstrated in ablated females in a number of species (Primavera 1985) Murugadass et al. (1988) reported that ablated M malcolmsonii carried more clutches of eggs and that there was an increase in the number of eggs per clutch Egg production in ablated series was double in comparison to unablated control Choy (1987) reported lower fecundity in ablated P canaliculatus

The number of larvae produced depends on the factors like female size fertility of males and occular ablation (Holtschmit and Remero 1991) While eyestalk ablation leads to predictable maturation and spawning it has been reported that ablation of captivity reared females results in reduced hatch rates (Lumure 1979 Emmerson 1980 Primavera and Posodas 1981) Employing different types of ablation Makinouchi and Primavera (1987) noticed that the hatching rates of unablated P indicus were significantly higher than females ablated by pinching the eyestalk but not from those ablated by tying and cautery The inconsistent quality of eggs particularly with successive spawnings has been attributed to the abnormal rapidity of maturation and overstimulation following ablation This decline in egg quality limits the reproductive life of ablated specimens necessitating the replacement of broodstock few weeks after ablation (Aquacop 1975 Beard and Wickins 1980 Simon 1982 Primavera 1983)

Browdy and Samocha (1985a) did not find any reduction in the hatch rates in subsequent spawnings within an intermoult period and concluded that a single mating is sufficient to fertilize four spawnings in P semisulcatus The number of larvae produced is known to have increased by the synchronization of moult cycle and reproductive cycle Hatching success between ablated and unablated treatments did not vary in P vannamei (Chamberlain and Lawrence (1981a b) P stylirostris (Chamberlain and Lawrence 1981b) and P semisulcatus (Browdy and Samocha 1985) Decrease in hatching success for successive spawning has been observed for ablated P monodon (Primavera and Boroglan 1979 Emmerson 1983) and P indicus (Emmerson 1980) while no such change was evident in unablated females

According to Browdy and Samocha (1985a) deterioration in sperm quality described by many authors is a direct result of worsening condition of animals over time and is not an effect of ablation

The extend to which females can remature is important in terms of recycling spawners Unilaterally ablated female prawns repeatedly mature and spawn viable eggs (Aquacop 1979 1982 Lumure 1979 Brown et al. 1980 Lawrence et al. 1980 Beard and Wickins 1980)

Eyestalk 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 _Metapeneaus 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-Trujillo and Lawrance 1991) Ablated _P vannamei_ produced larger spermatophore with more than two fold increase in sperm count over controls with no apparent effect on sperm quality (Leung Trujillo and Lawrence 1985) Bilateral ablation further enhanced gonad weight spermatophore weight and spermatophore count with the exception of sperm count (Leung Trujillo and Lawrence 1985) Chamberlain and Lawrence (1981a) reported a high mating frequency in tanks containing unilaterally ablated males compared to unablated _P vannamei_ These observations suggest the usefulness of eyestalk ablation in males also

III MATERIALS AND METHODS

3 1 Source of animals

M. equidens of various sizes were collected from Panangad area of Cochin backwaters near the College of Fisheries Cochin during the period March 1992 May 1993 Animals of 40 60 mm size were selected and maintained in 1 ton fibre glass tanks kept in the Macrobrachium hatchery at a salinity of 18 ppt Aeration was given from an air compressor through an air stone placed in the centre of the tank The day time temperature ranged from 25 5 to 28 ^{o}C

3 2 Feed formulation

The prawns were fed with a formulated diet prepared as per the composition of Sherief (1987) following the methods of Jayaram and Shetty (1981) using ingredients such as clam meat(40%) rice bran (25%) ground nut oil cake (25%) and tapioca flour (10%)

The biochemical composition of the feed is as follows

Protein	45 0%
Fat	4 0%
Ash	8 2%
Moisture	6 5%

3 3 Feeding

Feeding was done <u>ad libitum</u> the food being kept in a bowl placed in the tank Feeding was done using pre weighed feed in the evening hours after collecting the feed remains if any The feed remains where dried in an oven at 60 ^{o}C overnight before weighing

3 4 Eyestalk ablation

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

3 5 Experimental procedure

3 5 1 Experiment to study effect of unilateral and bilateral eyestalk ablations at different salinities on survival , growth and food conversion

Four prawns of size 40 60 mm each were introduced into 24 experimental units consisting of fibre glass tanks of 75 litre capacity Three treatments unablated unilaterally ablated and bilaterally ablated 8 tanks for each treatment were used Four salinities 5 1Ø 15 and 2Ø ppt were tried two units each for the three treatments

Survival was monitored daily and growth assessed weekly Actual food consumption was quantified by feeding each treatment with pre-weighed food from which the weight of feed remnance was deducted The experiment was carried out for 35 days

3 5 2 Experiment to study the effect of eyestalk ablation on ovarian devolopment, egg production and incubation period

Females were individually reared in 16 numbers of 75 litre capacity fibre glass tanks at a salinity of 2Ø ppt Unilateral ablation was performed in 8 of them the other 8 serving as control Observation on ovarian development was made regularly once in three days and a male introduced 6 days after female moulting in such tanks where female has adequate ovarian development The introduction of males was delayed to avoid the cannibalism of a soft shelled one by a unfamiliarised hard shelled one Cannibalistic tendency was found to be reduced by familiarization Spawning production of fertilized and unfertilized eggs and duration of moult cycle in each case were noted Fecundity was assessed at the second spawning of fertilized eggs and absolute fecundity calculated

3 5 3 Experiments to study the effect of ablation on growth, devolepment of androgenic gland, yas deferens, second cheliped and appendix masculina

Two experiments (expts 3 and 4) were conducted to study the effect of destalking on growth and reproduction in males

3 5 3 1 Effect of destalking on growth and cheliped development

16 males were used for the experiment (Expt 3) done during July to September 1992 They were reared individually and of these 8 were destalked unilaterally the other 8 serving as control Individual body growth and the development of cheliped were noted The experiment was carried out for 45 days

3 5 3 2 Effect of eyestalk ablation on the development of androgenic gland, vas deferns and appendix musculina

22 males were used for the experiment (expt 4) conducted for 25 days Of these 11 were unilaterally destalked the other 11 forming controls The measurement of the biomass of

the androgenic gland width of vas deferens and the length of appendix masculina was done from camera lucida diagrams of these after sacrificing and dissecting the prawns at the end of the experiment

3 6 Determination of water quality parameters

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

Salinity	Refractometer
Dissolved oxygen	Standard winklers method (Strickland and Parsons 1972)
pH	pH meter
Temperature	Thermometer

3 7 Evaluation Methods

3 7 1 Survival rate

3 7 1 1 Percentage survival rate

$$\frac{\text{Number of test animals harvested} \times 100}{\text{Number of test animals introduced}}$$

3 7 2 Growth

3 7 2 1 Percentage growth

$$\frac{(\text{Final length/weight Initial length/weight}) \times 100}{\text{Initial length/weight}}$$

3 6 7 2 Specific growth rate

$$S\ G\ (g/day)\ \ 3\ \frac{(wt^{33}\quad wo^{33})}{t\quad to}$$

3 7 2 3 Average daily percentage gain

$$ADPG = \frac{[\ln Wt\quad \ln Wo]}{t\quad to} \times (100)$$

3 7 2 4 Food conversion ratio

$$= \frac{\text{Wt of food consumed}}{\text{Wet weight gain}} \times 100$$

3 7 2 5 Moulting ovarian devolpement Incubation period

Number of spawns and days interval

3 7 2 6 Chela length

Percentage gain in 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 8 Statistical design and analysis

The first experiment (3 5 1) was conducted in factorial arrangement with randomized block design The other three experiments were conducted in completely randomised design The factorial experiment was conducted with three levels of treatments (viz unilateral ablation bilateral ablation and control) and four levels of salinities Each block thus consisted of 12 treatments (3 levels of treatments X 4 levels of salinities) Each treatment was replicated twice one in each block The experiment of unilateral eyestalk ablation of females (3 5 2) consisted of two treatments (unilateral ablation and control) each replicated 8 times The first experiment of unilateral eyestalk ablation of males (3 5 3 1) consisted of 2 treatments (unilateral ablation and control) each replicated 8 times The second experiment of unilateral eyestalk ablation using males also consisted of two treatments (unilateral ablation and control replicated 11 times each

3 8 2 Analysis of results

In the first experiment (3 5 1) in bilaterally ablated treatment all but one prawn died by the end of 14th day and hence only observations on survival could be obtained The degrees of freedom of error sum of squares on final percentage growth observation got reduced to 8 due to this However by analysing weekly specific growth rate and average daily percentage gain this was rectified Weekly observations were considered as replicates Theoretically the observations among weeks in the same tank were not independent but if one assumes that the interaction between weeks were the same for all treatments ANOVA could be a reasonable technique of measuring difference among treatments Angular transformations were effected to the survival data before analysis Further the comparison using ANOVA (Snedecor and Cochran 1967) was not possible due to the negative growth rate recorded in certain treatments during certain weeks Hence ANOVA was used for comparison, after correcting these negative values by uniformly adding a constant

Food conversion ratio could not be calculated for certain weeks due to the negative values of growth To facilitate comparison these values were predicted using missing plot technique and logarithmic transformation effected before analysis

ANOVA was used to compare the data on ovarian development and incubation period Logarithmic transformation was effected to the absolute fecundity data before analysis

The growth in ablated and unablated prawns and the percentage growth of chela was compared using ANOVA The data on the length and size of androgenic gland width of vas deferens length of appendix masculina and the number of spines on the appendix masculina were compared using ANOVA for ablated and unablated male prawns

IV RESULTS

4 1 Experiment to evaluate the effect of unilateral and bilateral eyestalk ablations on survival, growth and food conversion of prawns reared at different salinities

A factorial experiment conducted using randomized block design to evaluate the effect of eyestalk ablation at different salinities on a mixed population of M equidens showed that there is a significant effect for destalking on survival and food conversion Both final and intermediary data were analyzed for survival growth and food conversion

4 1 1 Effect of eyestalk ablation on survival at different salinities

As could be seen from table 3 the percentage survival rate in unablated treatement was 68 75 at the end of five weeks The reduction in the number of prawns was abrupt both in unilaterally and bilaterally destalked cases the percentage survival being 56 25 and 25 Ø respectively at the end of the first week By the 21st day 96 875% mortality occured in those destalked bilaterally the corresponding figures for unilateral destalking being only 56 25% (see fig 1)

Table 3 Survival of unilaterally and bilaterally eyestalk ablated and unablated M. equidens in percentage

Treatment	Days	Ø	7	14	21	28	35
Unablated		1ØØ	93 75	81 25	75	68 75	68 75
Unilaterally abl		1ØØ	56 25	56 25	43 75	4Ø 625	4Ø 625
Bilaterally abl		1ØØ	25	25	3 125	3 125	Ø

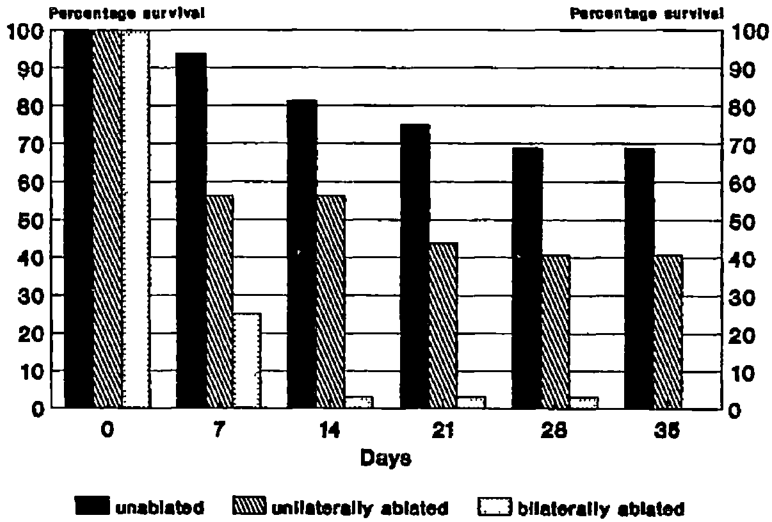


FIG I SURVIVAL OF UNILATERALLY AND BILATERALLY ABLATED PRAWNS

The average weekly percentage survival of unablated, unilaterally ablated and bilaterally ablated cases were 81 25 ±

11 9678 56 25 ± 20 6502 and 22 3958 ± 39 651 respectively On comparison these values are seen differing significantly at 5% level (Table 4) The mean weekly survival rates of all treatments put together ranged from 37 5 ± 26 885 to 58 33± 28 51

Table 4 Analysis of variance of data on average survival rates of eyestalk ablated and unablated prawns reared at different salinities

Source	Df	SS	MSS	F
Weeks	5	7099 2407	1419 8481	11 4087
Treatments	2	5437 6421	2718 820	21 8462
Error	10	2209 2030	220 9203	
Total	17	21208 7700		

Since the analysis showed significant variation pairwise comparison was made between the different salinity treatment means and the average weekly observations using critical difference

C D of treatments 19 1193

C D of survival rates 27 038

Comparison based on critical difference

Treatment	unablated	unilaterally ablated	bilaterally ablated
	81 25	56 25	22 92

Week	I	II	III	IV	V
	100	58 33	46 88	37 5	37 5

Connected lines show insignificant variation The survival rates significantly differed among ablation treatments However significant reduction in survival rate occurred only during the first week

It could be seen from the data (table 5 and fig II) that ablation significantly reduced survival rates The final percentage survival in unablated treatments at different salinities ranged from 50 to 100 with an average of 68 75 ± 16 5359 In unilaterally ablated treatment the survival rates ranged from 25 75 with an average of 40 625 ± 17 3993 Complete mortality was recorded in bilaterally ablated treatments (Table 5)

Table 5 Survival of eyestalk ablated and unablated prawns under different salinities after a period of five weeks

Salinities	5	10	15	20
Unablated	50 50	75 75	75 50	75 100
Unilaterally ablated	25 50	25 50	25 75	25 50
Bilaterally ablated	0	0	0	0

As could be seen from table 5 the final percentage survival at 5 ppt ranged from Ø 5Ø with an average of 37 5 ± 31 4576 at 1Ø ppt it ranged from Ø 75 with an average of 37 5 ± 31 4576 the range being Ø 75 with an average of 37 5 ± 31 4676 at 15 ppt and from Ø 1ØØ with an average of 41 6667± 37 2678 at 2Ø ppt These values do not differ significantly The interaction between ablation and salinity was not significant (vide table 6)

FIG 11 EFFECT OF ABLATION ON SURVIVAL UNDER DIFFERENT SALINITIES

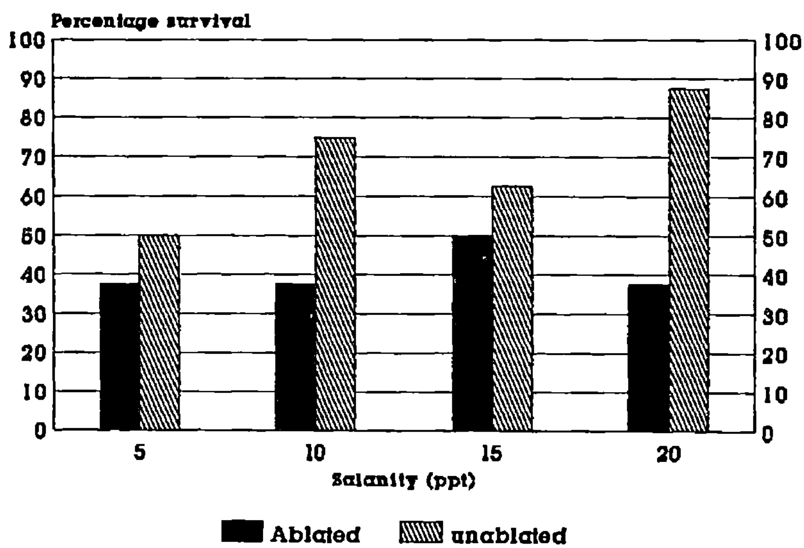


Table 6 Analysis of variance of the data on final percentage survival of eyestalk ablated and unablated prawns

Source	df	S S	M S S	F
Salinity	3	494 793	164 9310	0 7037
Ablation	2	19114 58	9557 2920	*40 7778
Interaction	6	1302 082	217 0137	
Error	12	2812 5	234 375	
Total	23	23723 96		

Since analysis of variance showed significant variation among survival rates of different treatments pairwise comparison was conducted between treatment means

CD for treatment means 16 6795

comparison based on CD

Treatment	Unablated	Unilateral abl	Bilateral abl
	68.75	40.625	0.000

The final percentage survival rates significantly differed between different ablation treatments

4 1 2 Effect of eyestalk ablation on growth

4 1 2 1 Effect of eyestalk ablation on final percentage gain in length

The percentage gain in length of unilaterally ablated treatment did not show any significant variation between ablated and unablated treatments Complete mortality was observed in bilaterally ablated treatment by the end of the experiment and hence only data on unilateral ablation could be obtained for growth studies. The percentage gain in length ranged from 1 25 to 25 5814 with an average of 11 7454 ± 7 0223 in ablated ones whereas in unablated treatment these values were lower ranging from 5 4187 to 18 1347 with an average of 8 5799± 3 9048 (vide table 7)

Table 7 Percentage gain in length of eyestalk ablated and unablated prawns reared at different salinities

Treatment	5	10	15	20
Ablated	1 25	14 2857	25 5814	17 1171
	7 7519	10 4149	5 441	12 1212
Unablated	9 9462	6 6964	9 308	5 4187
	6 25	6 1433	6 7416	18 1347

Table 8 Analysis of variance of data showing the difference in final percentage growth of eyestalk ablated and unablated prawns reared at different salinities

Source	d f	S S	M SS	F
Salinity	3	109 2733	36 4244	0 8697
Treatment	1	40 0822	40 0822	0 9577
Interaction	3	72 1532	24 0511	0 57
Error	8	335 0518	41 8815	
Total	15	556 5605		

The interaction between ablation and salinity is not significant The final percentage growth between ablation treatments and between salinities did not vary significantly (vide table 8)

4 1 1 2 **Effect of eyestalk ablation on percentage gain in weight under different salinities**

The percentage gain in weight of ablated and unablated *M equidens* did not differ significantly

The percentage gain in weight of unilaterally ablated treatment ranged from 1 3223 to 21 7734 with an average of 11 0177 ± 7 1601 In unablated treatments these values ranged from 2 4828 to 25 0095 with an average of 14 4420 ± 7 8699 The values differed insignificantly

Table 9 Percentage growth (in weight) at different salinities of eyestalk ablated and unablated prawns

	Salinity			
	5	10	15	20
Ablated	7 4381	1 3223	0 8969	15 7929
	21 7734	13 6098	9 0828	18 225
Unablated	21 5000	5 1309	22 3980	2 4828
	17 7479	11 3516	9 9150	25 0095

Table 10 Analysis of variance of the data on final percentage growth (in weight) of eyestalk ablated and unablated prawns reared at different salinities

Source	d f	SS	MSS	F
Salinity	3	218 6680	72 8893	1 0181
Ablation	1	46 9031	46 9031	0 6552
Interaction	3	114 2202	38 0734	
Error	8	572 7303	71 5913	
Total	15	952 5215		

The percentage gain in weight at different salinities and different ablation treatments did not vary significantly Their interaction is also not significant (Table 10)

4 1 2 3 Effect of eyestalk ablation on average specific growth rate

The average specific growth rates during five weeks ranged from 0 0035 to 0 0128 in unablated with an average of 0 0054 ± 0 0042 In ablated treatment specific growth rate ranged from 0 0061 to 0 0187 with an average of 0 0025 ± 0 0081 (Table 11)

Table 11 Average specific growth rate of eyestalk ablated and unablated prawns reared at different salinities

	Salinities			
	5	10	15	20
Control				
7	1 0153	1 0051	1 0051	1 0660
14	1 0076	1 0058	1 0078	1 0024
21	1 0070	1 0049	1 0035	1 0053
28	1 0045	0 9965	1 0054	1 0003
35	1 0079	1 0057	1 0128	1 0088

Ablated				
7	1 ØØ38	Ø 9992	1 ØØ24	1 Ø187
14	1 ØØ84	Ø 9984	Ø 9992	Ø 9789
21	Ø 9981	Ø 9939	1 ØØ42	1 ØØ179
28	1 ØØ31	1 ØØ1Ø	1 ØØ7	Ø 9998
35	1 ØØ41	1 ØØ55	1 ØØ39	1 ØØ94

A constant of 1 is added to each value to correct the negative values in the text

Table 12 Analysis of variance of the data on specific growth rate in eyestalk ablated and unablated prawns reared at dif ferent salinities

Source	d f	SS	mss	f
Salinity	3	Ø ØØØ1Ø3	Ø ØØØØ34	Ø 7234
Ablation	1	Ø ØØØØ84	Ø ØØØØ84	1 7872
Interaction	3	Ø ØØØØ42	Ø ØØØØ14	Ø Ø3
Error	32	Ø ØØ15Ø3	Ø ØØØ47	
Total	39	Ø ØØ17		

The aveiage specific growth rate in weight did not differ significantly between the four salinities and between ablated and unablated treatments (Table 12)

4 1 2 4 Effect of eyestalk ablation on average daily percentage gain in weight

The average daily percentage gain in weight ranged from -0 02901 to 1 4636 with an average of 0 4674 ± 0 04178 for unablated treatment and from 1 0794 to -1 7039 with an average of 0 2965± 0 5956 for ablated treatment

Table 13 Average daily percentage gain in weight of eyestalk ablated and unablated prawns reared at different salinities

	Salinity (ppt)			
Days	5	10	15	20
Control				
7	3 4636	2 4245	2 4245	2 5101
14	2 7120	2 4850	1 7997	2 1983
21	2 6448	2 0802	2 3159	2 4389
28	2 4091	1 7099	2 4853	2 0276
35	2 7105	2 4690	3 1203	2 7201
Ablated				
7	2 3764	1 9247	2 2401	3 7039
14	2 0213	1 8593	1 9176	0 9206
21	1 8164	2 5497	2 4268	3 6409
28	2 2918	2 0907	2 0676	1 9830
35	2 3976	2 4903	2 3896	2 8318

A constant of 2 is added to each value to correct the negative values to facilitate ANOVA Original values are quoted in the text

Table 14 Analysis of variance of the data on weekly average daily percentage weight gain in weight of eyestalk ablated and unablated prawns

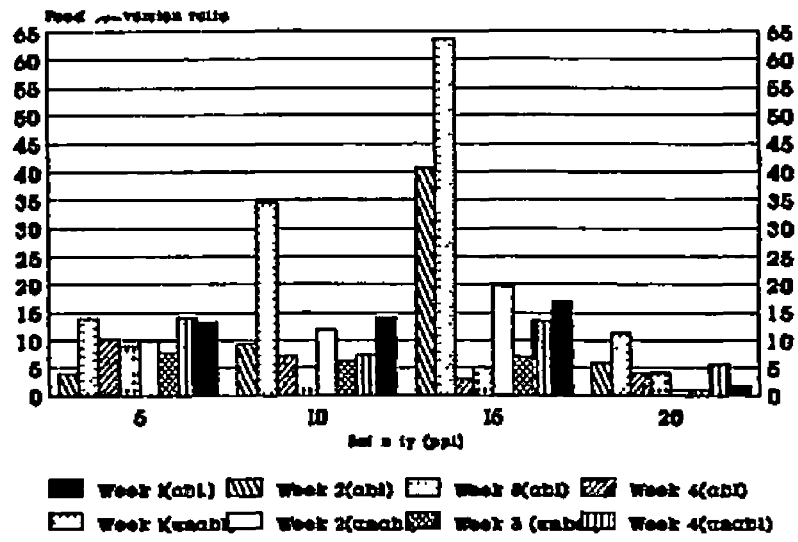
Source	df	SS	MSS	F
Salinity	3	Ø 7182	Ø 2394	Ø Ø595
Ablation	1	1 25Ø2	1 25Ø2	Ø Ø998
Interaction	3	Ø 4867	Ø 1622	Ø 45
Error	32	11 5935	Ø 3623	
Total	39	14 ØØ3		

The average daily percentage gain in weight did not vary significantly between ablation treatments and among salinities Their interaction also was not significant

4 1 3 Effect of eyestalk ablation on food conversion ratio

The data of the experiment on the effect of eyestalk ablation on food conversion ratio given in table 19 and depicted in figures III and IV show that eyestalk ablation has brought in significant variation in food conversion ratio The different salinities also produced significant variation in food conversion Food conversion ratio in unablated treatment pooled together ranged from 1 7481 to 63 7Ø36 with an average of 15 8533 ± 16 1816 and from 1 Ø11 to 19 629 with an average of 8 ØØ67 ± 4 7337 in ablated treatment

FIG III WEEKLY FOOD CONVERSION RATIO
OF ABLATED AND UNABLATED PRAWNS



FIGIV FOOD CONVERSION OF ABLATED AND
UNABLATED PRAWNS AT DIFFERENT SALINITIES

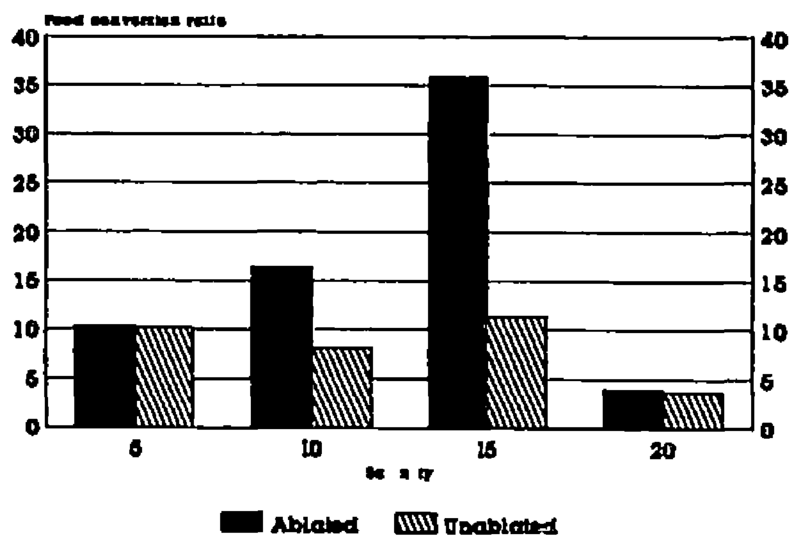


Table 15 Food conversion ratio of eyestalk ablated and unablated prawns reared at different salinities

	Salinities			
	5	10	15	20
Unablated				
Week				
I	13 1185	14 0866	16 9200*	1 7481
II	3 9636	9 3645	40 7655	5 8301
III	13 7940	34 5383	63 7036	11 3553*
IV	10 2220	7 71721	3 0579	4 0131
Ablated				
I	8 9128	5 1389	5 1505	4 2138
II	9 9006	5 1389	5 1505	4 2138
III	7 6454	6 1094*	6 9417	1 0111*
IV	14 0358	7 2809	13 4711	5 6771

* Values predicted using missing plot technique Growth obtained during this period was negative due to moulting or shedding of egg mass

At 5 ppt salinity level food conversion ratio ranged from 3 9636 to 13 7940 with an average of 10 2745 ± 03 8828 for unablated treatment and from 7 6454 to 14 0358 with an average of 10 1237 ± 2 3966 for ablated treatment At 10 ppt it ranged from 7 1721 to 34 5383 with an average of 16 2904 ± 10 8277 for unablated and from 5 1389 to 11 8675 with an average of

8 Ø958 ±2 8Ø67 for ablated The range noted vary from 63 7Ø36 with an average of 35 8423 ± 25 ØØ2Ø for unablated and from 5 15Ø5 to 19 629 with an average of 11 2981 ± 5 72Ø5 for ablat ed at 15 ppt At 2Ø ppt these values ranged from 1 7481 to 11 3553 with an average of 3 8638 ± 1 1669 for unablated and from 1 Ø11 to 5 6771 with an average of 3 67Ø9 ± 1 8989 for ablated (vide table 15)

Table 16 Analysis of variance on the data on food conversion of eyestalk ablated and unablated prawns reared at different salinities

Source	df	SS	MSS	F
Salinity	3	1 7582	Ø 5861	5 4665*
Ablation	1	Ø 3563	Ø 3563	3 3229*
Interaction	1	Ø 1539	Ø Ø513	Ø Ø4825
Error	24	2 5731	Ø 1Ø72	
Total	31	4 8415		

As could be seen in table 16 eyestalk ablation significantly affected food conversion ratio Salinity levels also affected food conversion ratio significantly

4 2 Experiment to evaluate the effect of eyestalk ablation on ovarian development, egg production and incubation

The experiment to find the effect of ablation of eyestalk in female M equidens has shown that destalking has enhanced ovarian development in all units as evidenced by the number of spawns within 60 days and the total number of eggs spawned

Table 17 Incubation period of eyestalk ablated and unablated M. equidens

Control	17	16	14	16	17	15	16	15
Ablated	14	15	16	16	14	16	14	14

The number of spawns was 2 25 per female in the case of ablated ones compared to 1 625 in unablated case Unilaterally ablated females moulted within an average interval of 13± 0 57 days while unablated ones moulted with an average of 15 8 ± 0 569 when devoid of eggs in the brood pouch Presence of eggs in the brood pouch delayed moulting in ablated animals

The incubation period was not found to vary significantly (table 18) although there is a slight reduction in ablated ones The incubation period ranged from 14 to 17 days in con trol with an average of 15 75 ± 0 903 while in ablated treat ment these values ranged from 14 to 16 days with an average of 14 875 ± 0 927

Table 18 Analysis of variance of data on incubation period of prawns under eyestalk ablated and unablated treatments

Source	df	SS	MSS	F
Ablation	1	3 0625	3 0625	2 9829
Error	14	14 3750	1 0268	
Total	15	17 4375		

The eggs hatched out into viable larvae in both ablated and unablated treatments although further study on the larvae was not carried out The unfertilized eggs were found to fall off within two days

The fecundity estimated by weighing the female and counting the number of eggs on the pleopods given in table 19 and figure V show that fecundity of unablated treatments ranged from 1508 to 3019 with an average of 22 11 75 ± 594 15 while it varied from 1318 to 3957 with an average of 2070 ± 602 6661 in ablated treatment

Table 19 Fecundity and absolute fecundity of eyestalk ablated and unablated prawns

a Fecundity

Control	1508	2810	3019	1620	1646	2841	1780	2470
Ablated	2957	1318	1971	2639	2806	1625	1830	1420

b Absolute fecundity

Control

300 998	1008 2162	899 2077,	790 7840
709 9112	831 8206	813 1567	837 7709

Ablated

1246 8379	1133 7634	795 7206,	755 1219
730 4438	774 47	947 2050	1199 9324

FIG V FECUNDITY OF ABLATED AND UNABLATED M EQUIDENS

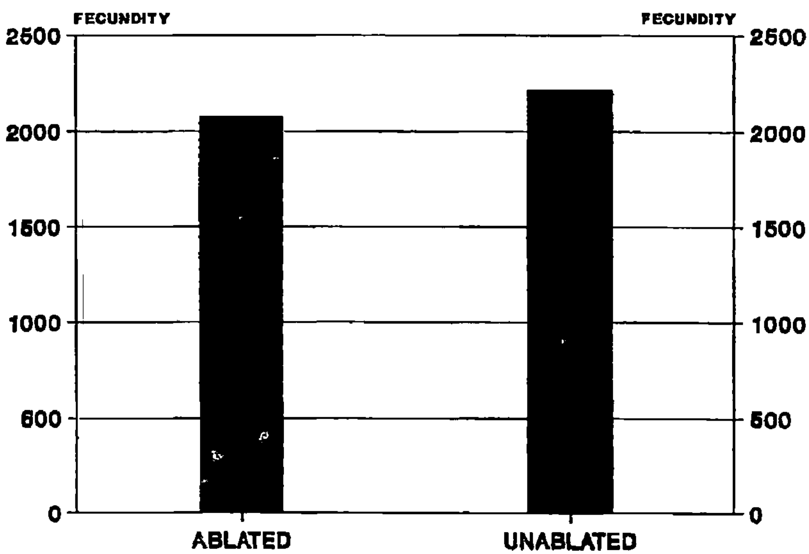
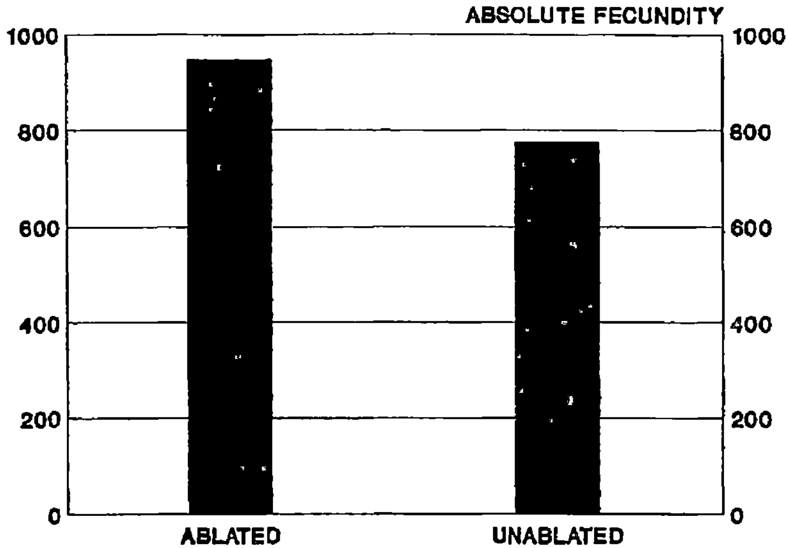


FIG VI ABSOLUTE FECUNDITY OF ABLATED AND UNABLATED M.EQUIDENS



The absolute fecundity ranged from 300 988 to 100 2162 with an average of 773 9832 ± 196 0859 for unablated and from 730 4438 to 1246 8379 with an average of 947 9368 ± 201 5927 for ablated thus showing an increase although not significant (vide table 19 and figure VI) These data were analysed using ANOVA

Table 2Ø Analysis of variance of data on the fecundity of eyestaik ablated and unablated treatments

Source	df	SS	MSS	F
Ablation	1	79524 ØØ	79524	Ø 1913
Error	14	5729769 ØØ	4Ø9269	
Total	15	753753 71		

As could be seen in table 2Ø the fecundity of ablated and unablated treatments did not vary significantly

Table 21 Analysis of variance of data on the absolute fecundity of eyestalk ablated and unablated treatments

Source	df	SS	MSS	F
Ablation	1	121Ø39 43	121Ø39 43	2 6782
Error	14	632714 28	45193 87	
Total	15	753753 71		

The absolute fecundity data of ablated and unablated treatments analysed and given in table 21 did not show any significant variation

The production of eggs was found to decrease with successive moults ın both ablated and unablated ones

4 3 Experiments to evaluate the effect of eyestalk ablation of males on growth, development of androgenic gland, vas deferens, second cheliped and appendix masculina

4 3 1 Effect of eyestalk ablation on body growth and cheliped development

The experiment conducted using male prawns having a size of 6 4 7 6 cms in body length and 3 Ø56 4 126 grams in weight has shown that there is a significant enhancement in body growth and cheliped development in ablated ones

A uniform survival rate of 87 5 percent was recorded in both ablated and unablated treatments

4 3 1 1 Percentage growth

The percentage growth of ablated males ranged from 5 6642 to 19 6Ø78 with an average of 11 4165 ± 4 6754 In unablated males these values ranged from 1 667 to 11 8644 with an average of 4 Ø794 ± 3 371 within a period of 45 days (vide table 22 and fig VII)

FIG VII GROWTH OF ABLATED AND UNABLATED MALE PRAWNS

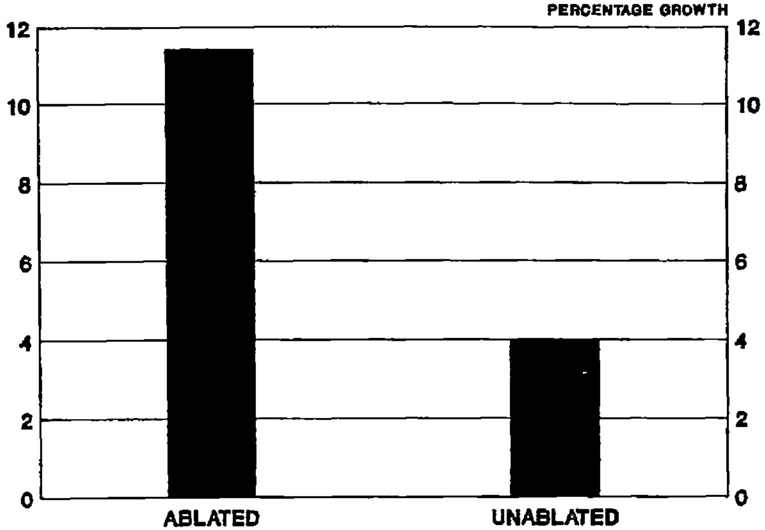


Table 22 Average percentage growth of eyestalk ablated and unablated male prawns

Ablated	5 6440	8 4746	16 000	19 6078
	6 7797	10 9091	12 5	
Unablated	1 6667	1 667	3 1250	11 8644
	5 0847	1 9231	3 2252	

On analysis (vide table 23) these values showed significant variation

Table 23 Analysis of variance of data on percentage growth in males of eyestalk ablated and unablated prawns

Source	DF	SS	MSS	F
Ablation	1	188 4134	188 4134	8 9118
Error	12	232 5603	21 1421	
Total	13	430 9764		

The percentage growth in ablated males was significantly greater than unablated ones

4 3 1 2 Cheliped development

The data on cheliped development of ablated prawns over control given in table 24 and figure VIII shows that there is significant difference The percentage growth in second cheliped length ranged from 8 333 to 28 2609 with an average of 13 2453 ± 6 7878 in ablated males and from 4 to 8 333 with an average of 5 9494 ± 14 065 in unablated ones

FIG VIII EFFECT OF EYESTALK ABLATION IN MALES ON DEVELOPMENT OF SECOND CHELIPED

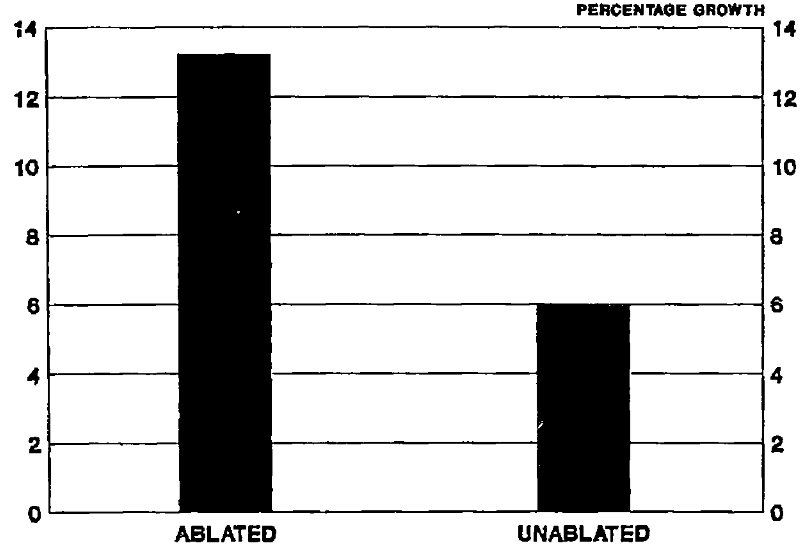


Table 24 Average percentage gain in second cheliped length in males of eyestalk ablated and unablated prawns

Ablated	10 3448	15 1020	8 3330	28 2609
	14 8387	8 8608	6 9767	
Unablated	4 6512	8 3330	5 5556	7 0423
	5 2174	4 000	6 8493	

On analysis using ANOVA these values showed significant variation (vide table 25)

Table 25 Analysis of variance of data on percentage growth of second cheliped in males of eyestalk ablated and unablated prawns

Source	df	SS	MSS	F
Ablation	1	186 3	186 3	6 6465
Error	12	336 3575	28 Ø296	
Total	13	522 6617		

As could be seen in table 25 ablation significantly increased the percentage growth in cheliped length in males

4 3 2 Effect of eyestalk ablation on development of androgenic gland, vas deferens and length of appendix masculina

Significant variation was not observed on hypertrophy of androgenic gland vas deferens and appendix masculina among ablated and unablated treatments

The range and standard deviation of body length cheliped length appendix masculina number of spines in the appendix masculina length of androgenic gland and width of vast defer ens are given in table 26

Table 26 Size of animals, chela length, length of appendix masculina, length and biomass of androgenic gland and width of vast deferens of eyestalk ablated and unablated prawns

		Range	Mean	SE
1	Size of the animal	**620-940**	**798 75**	**100 9254**
		800 890	850 00	30 3315
2	Chela length	**710-1311**	**1059 33**	**218 4312**
		570 1370	1140 00	330 0000
3	Length of appendix masculina	**0 1420-0 1854**	**0 1670**	**0 01630**
		0 1595 0 2005	0 1825	0 01524
4	No of spines on appendix masculina	**24-40**	**35 1875**	**4 2715**
		27-43	33 5000	5 3292
5	Length of androgenic gland	**0 1667-0 3000**	**0 2523**	**0 0433**
		0 2133 0 3083	0 2760	0 0351
6	Width of vas deferens	**0 0792-0 1268**	**0 0943**	**0 0195**
		0 0467 0 1725	0 1042	0 0465
7	Biomass of androgenic gland	**0 00044-0 00112**	**0 00078**	**0 000280**
		0 00041 0 00054	0 00046	0 000051

ablated
unablated

These parameters under ablated and unablated treatments were compared using ANOVA as could be seen in the following tables

Table 27 Analysis of variance of data on the length of appendix masculina of eyestalk ablated and unablated prawns

Source	df	SS	MSS	F
Ablation	1	0 0007	0 0007	2 3333
Error	11	0 0033	0 0003	
Total	12	0 0046		

The length of appendix masculina did not vary significantly between ablated and unablated treatments (vide table 27)

Table 28 Analysis of variance of data on the number of spines on the appendix masculina of eyestalk ablated and unablated prawns

Source	df	SS	MSS	F
Ablation	1	8 7621	8 7621	0 3347
Error	11	279 2019	26 1789	
Total	12	287 9680		

So also the number of spines in the appendix masculina did not vary significantly between ablated treatments (vide table 28)

Table 29 Analysis of variance of data on the length of androgenic gland of eyestalk ablated and unablated prawns

Source	df	SS	MSS	F
Ablation	1	Ø ØØ18	Ø ØØ18	Ø 9497
Error	11	Ø Ø211	Ø ØØ19	
Total	12	Ø Ø229		

The length of androgenic gland did not vary significantly between ablated and unablated treatments (vide table 29)

Table 3Ø Analysis of variance of data on biomass of androgenic gland of eyestalk ablated and unablated prawns

Source	df	SS	MSS	F
Ablation	1	Ø ØØØØØØ262	Ø ØØØØØØ262	1 696
Error	11	Ø ØØØØØ1699	Ø ØØØØØ154	
Total	12	Ø ØØØØØ1961		

The biomass of the androgenic gland did not vary significantly between ablated and unablated treatments (vide table 3Ø)

Table 31 Analysis of variance of data on the width of vas deferens in eyestalk ablated and unablated prawns

Source	df	SS	MSS	F
Ablation	1	0 0007	0 0007	2 3333
Error	11	0 0033	0 0003	
Total	12	0 0040		

The width of the vas deferens did not vary significantly between ablated and unablated treatments (vide table 31)

V DISCUSSION

5 1 Effect of unilateral and bilateral eyestalk ablation on survival

In the present investigation the experiment conducted to evaluate the effect of ablation on survival revealed that survival rates varied significantly among the various ablation treatments Significantly high mortality was observed during the first week and these were mostly associated with moulting Higher mortality prior to moulting is likely to be the result of shock of operation as well as physiological stress imposed on prior to ecdysis(Lockwood 1968)

Eyestalk ablation although used as a means for inducing gonadal maturation in prawns is known to adversely affect the physiology of crustaceans resulting in high mortalities (Choy 1987) As has been observed in the present study the increment in number of moult with ablation can result in cannibalism of recently moulted prawns Similar observation was made by Choy (1987) in P canaliculatus wherein 15% and 12% mortality was observed in ablated and unablated treatments respectively

Among the ablation treatments tried in the present study unilateral ablation produced a survival rate of 40 5% in comparison to the complete mortality observed in bilaterally ablated treatment This shows that unilateral ablation is causing only a minor imbalance in the physiology of the prawn as suggested by Halder (1978) that removal of only one eye reduces the risk of mortality and impairment of normal responses towards feeding and mating Further removal of one eyestalk and its contents was observed by many authors to be sufficient to enhance gonadal development at the same time reducing the adverse effect of bilateral destalking in prawns such as P. aztecus (Aquacop 1975) P. indicus (Makinouchi and Primavera 1987) P. japonicus (Aquacop 1975) P merguiensis (Aquacop 1975) P monodon (Aquacop 1977 Primavera and Borlongan 1977) P orientalis (Arnstein and Beard 1975) and P semisulcatus (Aquacop 1975) However Alikunhi et al (1975) and Rajyalakshmi et al (1988) have found bilateral destalking necessary for ovarian development in P monodon In the present study bilateral destalking resulted in complete mortality by the fifth week (30th day) the majority succumbing by the 7th day itself(75 0%) It was observed that bilateral ablation caused loss of balance and swimming in spiral motion though feeding was not affected Similar observations were made by Caillouet (1973) in P duorarum Alikunhi et al (1975) in P merguiensis and P monodon and Arnstein and Beard (1975) in

P orientalis High/total mortality of bilaterally ablated prawns was observed in M lanchestri (Ponnuchamy et al 1981) and in M rosenbergii (Chakravarthy 1992) In the case of M rosenbergii Chakravarthy (1992) has attributed the total mortality of bilaterally destalked animals to the rapid increase in body size followed by the inadequate preparation for moult leading to failure in moult Cannibalism of recently moulted prawns by hard shelled ones is quite common in certain species of penaeids like P monodon (Varghese et al 1975) and Macrobrachium sp (Ling 1969) This has been found both in ablated and unablated the possibility of cannibalism is more in ablated ones as there is increasing moulting frequency in this category In the experiments to find the effect of eyestalk ablation on reproduction of females and males, wherein they were reared individually, better survival rate of 87 5 per cent was obtained The pale colouration after moulting which was observed in M rosenbergii by Chakravarthy (1992) was witnessed in the present study also

Bilateral ablation was reported to have resulted in four fold increase in mortality in the crab Paralithodes camtschatica (Molyneaux and Sheirley 1988)

Mortality associated with eyestalk ablation may be immediate or occurring subsequently after a period There are cases where ablation had no significant effect on survival (Aquacop 1977 Vincentre *et al* 1979) In *P monodon* (Primavera *et al* 1978) and *P kerathurus* (Lumure 1979) there was substantial mortality immediately after ablation In the present study in *M equidens* 100% mortality occured in bilaterally ablated treatment by the 30th day

In the present investigation ablation of eyestalks was done with electrocautery equipment which was found to be a comparatively better method by many workers such as Makinouchi and Primavera (1987) and Muthu and Laxminarayana (1979 1981) According to Muthu and Laxminarayana (1979) cauterization prevented bleeding and produce little mortality in *P indicus* and *P monodon* Browdy and Samocha (1985 a) attributed the good survival rate of ablated *P semisulcatus* partly to the use of electrocautery method which prevented bleeding and infection

5 2Effect of eyestalk ablation on growth

In the present investigation it was found that in a mixed population of *M equidens* growth neither in terms of length nor in terms of weight gain, was significantly affected by eyestalk ablation as evident from the data on percentage growth weekly specific growth and average daily percentage gain in length and weight But in another experiment of destalking done in males (vide 4 3 1) significant variation in growth was recorded

A marginal reduction in the intermolt period from an average of 15 8 +/ Ø 569 to 13+/ Ø 57 days was however observed in the present study even in the case of a mixed population The effect of this reduction in intermoult period has not reflected on growth as there was no significant variation in growth Many investigators working on Palaeomonid prawns have obtained better growth by way of destalking In the case of M rosenbergii growth rate was reported to be high in bilaterally ablated prawns low in unilaterally ablated and minimum in controls (Chakravarthy 1992) Pandian and Sindhukumari (1985) reported 1 6 times growth and 1 5 times growth efficiency in ablated M. nobilii Murugadass et al (1988) reported accelerated growth in destalked M. malcolmsonii in both sexes controlled males recording a growth of 227 KJ while destalked males a growth of 355 KJ In females the growth increment was 169 KJ in unablated female and 34Ø KJ in ablated treatments The sexual difference in response to growth by way of destalking observed by Murugadass et al (1988) in M malcolmsonii is in agreement to the present investigation wherein males had a better growth than the mixed population

The insignificant growth increment observed in mixed population of M equidens in the present study may be due to the fact that the females in the mixed population has responded to reproduction than growth

Murugadass et al (1988) attributed the increment in growth of ablated ones to the effect of less energy being channalised to exuvial production Ponnuchamy et al (1981) reported marked increase in the growth of bilaterally destalked M. lanchestri prawns (47 7 mg/prawn) as compared to those that were unilaterally destalked (19 9 mg/prawn)

Mostly in penaeids no significant difference in growth was found between ablated and unablated as seen in P monodon (Emmerson 1983) and P. semisulcatus (Browdy and Samocha 1985a) Primavera et al. (1982) reported that in terms of growth in male P indicus ablated and unablated groups showed similar trends They found that unablated females to be slightly bigger although not significant However in the case of P monodon Alikunhi et al (1975) reported bilaterally ablated ones reaching twice the body weight of unablated controls P canaliculatus responded to eyestalk ablation by increasing growth rate as a result of increased frequency and greater increment per moult (Choy 1987) Thus as suggested by

Wickins and Beard (1974) the increased production of eggs by most of the penaeid prawns as a result of destalking may be possibly the reason for not registering increased growth

Eyestalk ablation has always resulted in a significant positive growth in lobsters According to Radhakrishnan and Vijayakumaran (1984) destalking accelerated growth in Panulirus homarus which they attributed to the higher moulting frequency and higher percentage gain at each moult While faster moulting is attributed to the removal of MIH factors from the eyestalk the higher percentage weight gain at moult was presumed to be due to the elimination of the hormone that regulates water uptake during ecdysis (Carlisle 1955) Koch (1952) attributed the abnormal size attained by ablated crab Eriocher sinensis to increased accumulation of water and not to tissue synthesis However Mauviout and Castell (1976) reported real growth with a lower level of lipid deposition in muscle and hepatopancreas of ablated lobster Homarus americanus This indicates an accelerated growth of protein deposits and tissue synthesis in ablated animals In the case of the crab Paralithodes camshatica eyestalk ablation did not significantly change the growth or wet weight or carapace length (Molyneaux and Shierly 1988)

In the present investigation on M equidens no biochemical analysis was conducted to find out the response of destalking

5 3 Effect of eyestalk ablation on food conversion

Food conversion was found significantly reduced in ablated treatment in the present study showing better efficiency Marian *et al* (1986) observed similar results in *M lammarei* Thus destalking has been recommended in culture system in the case of prawn *M lammarei* (Marian *et al* 1986) and lobster *Homarus americanus* (Koshio *et al* 1990) to effect better feed efficiency

Eyestalk ablation in *M equidens* in the present study did not incapacitate the prawn from detecting food probably because of well developed chemo receptor system This observation in *M equidens* is contrary to the observations of Choy (1987) in *P canaliculatus* in which feeding was reported to have been adversly affected by ablation Radhakrishnan and Vijayakumaran (1984) reported that food conversion efficiency in *Panulirus homarus* was double in ablated treatments as compared to controls Kumari and Pandian (1987) reported an improvement in food conversion in *M nobilii* Significant improvement was effected by ablation in *H americanus* (Koshio *et al* 1990) Depletion of hepatopancreatic reserves was observed in *H americanus* by Aiken (1980) but the present investigation was not extended to such areas

5 4 Effect of eyestalk ablation on reproduction

5 4 1 Effect of eyestalk ablation on ovarian development, fecundity and incubation period

The results of the experiment conducted to study the impact of unilateral eyestalk ablation of reproduction in female M equidens indicated that eyestalk ablation enhanced ovarian development but the effect was insignificant in terms of fecundity and absolute fecundity There was some enhancement in moulting frequency also These results are in agreement with the known fact that eyestalk ablation in crustaceans enhances either precocious moulting or gonadal development (Adiyodi and Adiyodi 1970 Calliouet 1973 Arnstein and Beard 1975 Emmerson 1980 1983) The stimulation is known to depend on the relative interaction of the environmental factors as well as the age and moult stage of the animals operated upon (Bliss and Beyer 1964 Laubier 1978 Emmerson 1980 1983) In the present investigation it was observed that spawning is closely related to moulting and occurred always in the post moult period as observed by Shyama (1987) in M idella It could be seen from the data of the present study that in M equidens unilateral destalking brings about an enhancement in the moulting frequency In the case of incubation moult alone there is some delay may be because of the presence of eggs in the brood pouch which are to be hatched Shyama (1987) made similar observations in M idella Scudamore (1948) working on crayfish found that brooding induced prolonged production of MIH in X organ sinus gland complex Kamaguchi (1971) made similar observations in fresh water prawn P paucidens It may be assumed that the release of larvae might be responsible for stopping the production of MIH and accelerating MH activity

Fecundity and absolute fecundity did not show any significant variation among the ablated and unablated treat ments in the present investigation Though many studies on fecundity in Macrobrachiumspp are there (Ling 1969 Patra 1976 Malecha 1983 Coasta and Wanninayake 1986 Mathavan et al 1986 Ang and Kok 1991 Manna and Raut 1991 Udo and Epke 1991) the effect of eyestalk ablation on spawn size is poorly defined

Murugadass et al (1988) reported that M malcolmsonii carried more number of eggs per clutch and also that egg pro duction in ablated series was double when compared to control The shorter duration of the present investigation would have resulted in an insignificant fecundity change as the effect of ablation would be more pronounced only in the third or subsequent moult cycle when the prawns tend to enter neutor cycle

In penaeid prawns substantial increase in the number of eggs has been reported by eyestalk ablation Thus in P indi cus Primavera et al (1982) reported an increase of eight times and Emmerson (1980) reported a marginal increase in egg production In P schimitii Nascimento et al (1991) observed a significant increment in the number of eggs Santiago (1977) observed that the fecundity is comparable among ablated captive and wild females of P monodon Contrary to the above obser vations lower fecundity was observed in P canaliculatus (Choy 1987) Thus it can be seen that there is considerable variation in response as to the number of eggs produced among the different species some showing substantial increase some marginal and some others such as M equidens showing insignifi cant increase

A progressive decline in fecundity was noted in the present investigation similar to the results observed by Choy (1987) in P canaliculatus

Incubation period in M equidens differed insignificantly among ablated and unablated treatments and only a slight reduction was noted among ablated ones This lack of response of destalking on ıncubatıon period may be to allow normal development and hatching of the eggs carried in the brood pouch

5 4 2 Effect of eyestalk ablation on the development of androgenic gland, second cheliped, vas deferens and appendix masculina

In the experiment conducted to fınd the effect of unılateral destalkıng on male reproduction in terms of ıts effect on hypertrophy of androgenic gland and devolepment of second cheliped vas deferens and appendix masculina it was observed that except for the second chelıped the other features dıd not significantly differ between ablated and unablated treatments Androgenıc gland is known to secrete a hormone which determines the primary and secondary sexual characters (Thampy and John 1972 1973 Nagmine _et al_ 1980) and control behavioral sexual characters (Charniaux cotton 1960 1961) Sex and reproduction are under the direct control of the androgenic gland which in turn is under the control of X organ sinus gland complex The higher development of the second cheliped in the ablated treatment points to the fact that X organ sinus gland is keepıng the androgenic gland under check

The androgenic gland being a holocrine gland enlarges as and when there is an increase in 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 chelipeds the number of spines in the appendix masculina and the width of vas deferens there is a corresponding increase in the size of the androgenic gland Hypertrophy of androgenic gland after eyestalk ablation was reported in several crustaceans (Adiyodi and Adiyodi 1970) The shorter duration of the experiment and timing of the experiment towards the end of the breeding season would have resulted in insignificant variation in the size of the androgenic gland number of spines in the appendix masculina and width of vas deferens observed in the present investigation although there is a marginal increase in the biomass of the androgenic gland and the number of spines in the appendix masculina in the destalked prawns

In P monodon and P merguiensis Alikunhi et al (1975) found ablation to induce precoecious gonadal devolepment in immature males Ablation was found to increase gonadal size in P setiferus (Lawrence et al 1979)and increased gonad size and mating frequency in P vannamei (Chamberlain and Lawrence 1981) Leung Trujillo and Lawrence (1985) noted that unilateral eye stalk ablated P vannamei produced larger spermatophores 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 requirements may be involved in male reproduction (Leung Trujillo and Lawrence 1991) which was not investigated in the present study

VI SUMMARY

1 The present study in the prawn Macrobrachium equidens was taken up to evaluate the effect of eyestalk ablation in factors like survival growth and food conversion in a mixed population on ovarian development fecundity and incubation period of females and on the devolepment of androgenic gland second cheliped number of spines in the appendix masculina and width of vas deferens in males

2 In the first experiment conducted for a period of 35 days on a mixed population unilateral eyestalk ablation bilateral eyestalk ablation and control treatments were employed at four levels of salinities viz 5 10 15 and 20 ppt The results of this experiment revealed that eyestalk ablation significantly reduced survival rates in mixed population rearing but not in subsequent experiments when they were reared individually

3 In the first experiment complete mortality was observed in bilaterally destalked prawns by the 30th day the survival rate being 25% at the end of 7th day In the case of unilateral destalking the rate of survival at the end of 7th day was 56 25% At the end of 35 days a survival rate 40 625% was

recorded in unilaterally destalked treatment while it was 68 75% for the control Tne decline in survival rate was abrupt in bilaterally eyestalk ablated prawns while it was gradual in both unilateral and control treatments Different salinity levels tried viz 5 1Ø 15 and 2Ø ppt did not alter the survival rate signıficantly

4 An appreciably high survival rate of 87 5% was obtained when unilaterally destalked and unablated prawns were reared indıvidually in the experiments II III and IV showing that cannibalism would have contributed much in the lowering of survival rate

5 There ıs no significant influence of destalkıng on growth the final percentage gain in length rangıng from 1 25 to 25 5814 with an average of 11 7454 ± 7 Ø233 in ablated treatment in comparison to the values of 5 4187 to 18 1347 with an average of 8 5799 ± 3 Ø948 in unablated ones

6 Fınal percentage gaın ın weıght ranged from 1 3223 to 21 7734 with an average of 11 Ø177 ± 7 16Ø1 for ablated and 2 4828 to 25 ØØ95 wıth an average of 14 442Ø ± 7 8699 for unablated treatment

7 Comparison of the specific growth rate of destalked and control treatments shows that there is no significant difference The weekly specific growth rate in weight ranged from Ø ØØ35 to Ø Ø128 with an average of Ø ØØ54 ±Ø ØØ42 in unablated and -Ø ØØ61 to Ø Ø187 with an average of Ø ØØ25 ±Ø ØØ81 for ablated treatment

8 The values of average daily percentage gain in weight did not vary significantly between destalked ones and control It ranged from Ø 29Ø1 to 1 4636 with an average of Ø 4674 ±Ø 4178 in unablated treatment and 1 Ø794 ±1 7Ø89 with an average of Ø 2965 ±Ø 5456 for ablated

9 Eyestalk ablation significantly reduced food conversion ratio the range being 1 7481 to 63 7Ø36 with an average of 15 8533 for unablated and from 1 Ø11 to 19 629 with an average of 8 ØØ67 ±4 7337 in ablated treatment

1Ø Significant difference in food conversion was observed among different salinity levels it being better at 2Ø ppt

11 Ovarian devolepment was found to have enhanced by eyestalk ablation The number of spawns per female was 2 25 numbers in ablated treatment while it was only 1 625 in unablated treatment within a period of 6Ø days

11 Unilateral ablation resulted in a reduction in moulting interval to 13 ± Ø 57 days the interval being 15 8 ± Ø 579 for unablated prawns

12 The incubation period was not found to differ between ablated and unablated it being 15 57 ±Ø 9Ø3 in control and 14 875 ± Ø 927 in ablated

13 The fecundity and absolute fecundity values did not vary significantly between ablated and unablated treatments The average fecundity was found to be 2211 75 ± 594 15 for control and 2Ø7Ø ±6Ø2 661 for the ablated treatments Absolute fecundity value of 947 9368 ± 2Ø1 5927 for the ablated prawns was marginally higher when compared to the value of 773 9Ø32±198 Ø859 for control

14 The growth rate in males significantly increased with ablation the values being 11 4165 ± 4 675 in ablated in comparison to 4 Ø794 ± 3 371 for controls showing that in males the stored energy may be mobilized for growth more in comparison to females which had responded more favourably to reproduction

15 Unilateral eyestalk ablation was found to cause an increase in length of second cheliped of males significantly the

percentage increase being 13 2453 ± 6 7878 in ablated and 5 9494 ±14 Ø65 in control males

16 The length and biomass of androgenic gland length of appendix masculina the number of spines in the appendix masculina and the width of vas deferens did not show significant variation between ablated and unablated treatments although there was a marginal increase in the biomass of the androgenic gland and the number of spines in the appendix masculina

17 Thus it can be seen that the effect of destalking in females is more towards reproduction enhancing the ovarian devolepment and absolute fecundity in comparison to males where the influence on reproduction being only marginal enhancing the develepment of the androgenic gland and the secondary sexual characters such as the second cheliped and appendix masculina But in males contrary to the case in females there is a significant effect for destalking on growth

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EFFECT OF EYESTALK ABLATION ON GROWTH AND REPRODUCTION OF *MACROBRACHIUM EQUIDENS* (Dana)

By
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ABSTRACT OF A THESIS

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ABSTRACT

The objective of the present study is to evaluate the effect of eyestalk ablation on survival growth food conversion and reproduction of adult *Macrobrachium equidens*

In the first experiment conducted for 35 days unilateral eyestalk ablation bilateral ablation and control treatments were employed at four level of salinities viz 5 10 15 and 20 ppt Complete mortality in bilateral ablation and survival rates of 40 625 % and 68 75% for unilateral and control treatments were recorded at the end of the experiment the values ranging significantly The four salinity levels applied did not significantly affect survival

The difference in growth between ablated and unablated treatments was insignificant in terms of percentage gain specific growth rate and average daily percentage gain in terms of length and weight Salinity levels also showed insignificant difference in growth Food conversion rate was significantly better in ablated treatment The different salinity levels tried showed significant difference with regard to food conversion being better at 20 ppt

In the second experiment conducted for 60 days on females eyestalk ablation was found to enhance ovarian

In the second experiment conducted for 60 days on females eyestalk ablation was found to enhance ovarian development in terms of the number of spawns per female Fecundity absolute fecundity and incubation period were not significantly affected although a marginal increase in absolute fecundity could be observed

In the experiments conducted on males it was found that growth rate and cheliped devolepment were significantly better in ablated ones The number of spines in the appendix masculina length and biomass of the androgenic gland and width of vas deferens did not show significant variation although a marginal increase in the biomass of the androgenic gland and the number of spines in appendix masculina was observed in ablated treatment

The different eyestalk ablation experiments have shown that in females destalking does not stimulate growth but there is better response for reproduction whereas in males there is better growth and only marginal response to reproduction