INDUCED MATURATION OF *PENAEUS INDICUS* USING EXOGENOUS HORMONE

By

JOHNSON D' CRUZ, B.F.Sc.

THESIS

Submitted in partial fulfilment of the requirement for the degree

MASTER OF FISHERIES SCIENCE

Faculty of Fisheries

Kerala Agricultural University

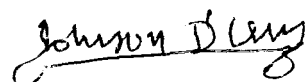DEPARTMENT OF AQUACULTURE

COLLEGE OF FISHERIES

PANANGAD, COCHIN

1995

IN LOVING MEMORY OF

MY BELOVED FATHER

PETER D'CRUZ

DECLARATION

I hereby declare that this thesis entitled "**INDUCED MATURATION OF PENAEUS INDICUS USING EXOGENOUS HORMONE**" is a bonafide record of research work done by me during the course of research and that the thesis has not formed the basis for the award to me of any degree, diploma, associateship, or other similar title, of any other University or Society.
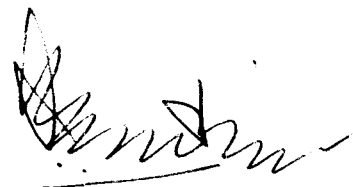
Panangad,
20/4/1995.

JOHNSON   D'CRUZ

CERTIFICATE

Certified that this thesis entitled **"INDUCED MATURATION OF PENAEUS INDICUS USING EXOGENOUS HORMONE"** is a record of research work done independently by Sri.**JOHNSON D'CRUZ** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to him.
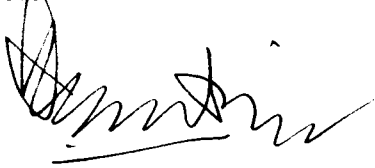
Panangad,
21/4/1995.

Dr. M.J. SEBASTIAN
(Chairman, Advisory Board)
Professor and Head,
Department of Aquaculture,
College of Fisheries,
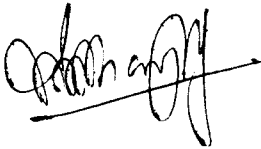Panangad,Kochi.

ADVISORY COMMITTEE

CHAIRMAN

Dr. M.J.SEBASTIAN

PROFESSOR AND HEAD,
DEPARTMENT OF AQUACULTURE,
COLLEGE OF FISHERIES,
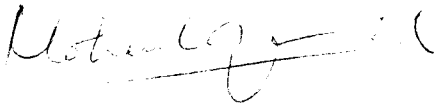PANANGAD,
KOCHI

MEMBER

Dr.D.M.THAMPY

PROFESSOR,
DEPARTMENT OF AQUACULTURE
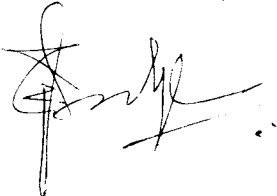COLLEGE OF FISHERIES,
PANANGAD, KOCHI

MEMBER

Dr. C.MOHANAKUMARAN NAIR

ASSISTANT PROFESSOR
DEPARTMENT OF AQUACULTURE,
COLLEGE OF FISHERIES,
PANANGAD, KOCHI.

MEMBER

Sri. T.M.SANKARAN

ASSOCIATE PROFESSOR AND HEAD,
DEPARTMENT OF MANAGEMENT STUDIES,
COLLEGE OF FISHERIES,
PANANGAD, KOCHI.

ACKNOWLEDGMENTS

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

I am deeply indebted to Dr. M. J. SEBASTIAN, Professor and Head, Department of Aquaculture, College of Fisheries, Panangad, for his valuable guidance and constant encouragement which were the motive forces behind the timely completion of my research work. I remain grateful to him for the helpful advice and constructive suggestions given during the course of the experiment and preparation of the thesis. His keen attention and advice helped me a lot in preparation of the thesis and submitting it in the present form. I am also ever thankful to him for he in the capacity of Dean made the procurement of material easy and provided all facilities necessary for the experiment.

It is with great sense of gratitude I remember the name of Dr. D. M. THAMPY, Professor, Department of Aquaculture, College of Fisheries, Panangad for I was fortunate to have benefited from the valuable counseling, inspiration and help from him in carrying out the research smoothly and in presentation of this written document.

My heartfelt thanks are due to Dr. C. MOHANAKUMARAN NAIR, Assistant Professor, Department of Aquaculture, College of Fisheries, Panangad, for being a constant source of support in my endeavours and also for his cordial and timely help during the course of the experiment and the thesis work.

CONTENTS

List of tables

.

List of plates and figures

Plates

Figures

I INTRODUCTION

The Indian white shrimp, *Penaeus indicus* has got great potential for commercial farming in India and elsewhere in Asia. It fetches higher market value than the commercially preferred *P.monodon* (Hirasawa, 1985; Seat and Carlos,1994). Now the trend is towards the changing over to the farming of *P.indicus* from *P.monodon*, as the former is more resistant to disease and suitable for high density stocking than the latter. It is an ideal species for culture in high saline areas and it is comparatively an easy-to-breed species (Primavera,1985). But the brooder availability from wild is seasonal and unpredictable.

Seed is an important input in shrimp farming. The major constraint to the development of the shrimp farming industry in India and elsewhere is the difficulty to procure quality seed at the right time and in sufficient numbers. Control over the reproductive cycle of an animal of commercial importance is an essential prerequisite for programming its culture (Lumare, 1979). The profitable production of any agriculture or aquaculture crop requires an abundant, reliable and inexpensive supply of seed. This seedstock must also be healthy and hardy enough to grow well under the intended culture conditions. Without such seedstock intensive and large scale crop production is not possible. Marine shrimp culture is now undergoing revolution similar to the one that has already been experienced by the terrestrial crops. Although some shrimp larvae are still collected from the wild, there is tremendous progress in hatchery production of seed, especially of the most desirable species (Anon., 1992).

Hatchery systems have been developed in various countries to meet the demands of quality shrimp seed. For effective hatchery operation, a steady supply of spawners of the desired species at the appropriate time is necessary. The procurement of ripe spawners for hatchery is a costly and uncertain operation in most of the shrimp farming nations. This has generated global interest in the induction of maturation of penaeid shrimp in captivity (Muthu and Laxminarayana,1982) which might form a consistent and easily available source of broodstock for regular spawning in commercial shrimp hatcheries. Controlling the reproduction in captivity could help to provide a reliable year-round supply of juveniles, serve in developing selective-breeding programmes and be generally useful for obtaining disease-free spawners (Yano,1992a).

It was in early 1970 s that the world heard of eyestalk ablation (ESA) technique which induced ovarian maturation in a penaeid shrimp, *P. duorarum* (Idyll,1971; Caillouet,1972). This was achieved almost three decades after the observation of Panouse (1943) that ESA results in precocious gonadal development in *Palaemon serratus*. Since then, the studies on induced maturation of shrimps by the application of this method was tried by many. This was one of the important developments in aquaculture leading to the increased production of shrimp seed and commercial production of farmed shrimps, increasing its share of contribution to total shrimp production from less than 2 % in 1980 to over 25 % at present.

Though ESA continues to be synonymous with induced breeding of penaeids in captivity, it is a one way process, which switches breeding programme on, but there is no mechanism for turning it off. It also affects other physiological functions within the body of the animal with a result that eggs deteriorate with repeated spawning and as a consequence, each female breeder has a shorter productive life span. In addition to these short-comings other drawbacks like high mortality, lower fecundity and weak offspring also result following this cruel method of organ removal (Primavera,1985;Bray and Lawrence, 1992; Walker, 1994). In shrimp aquaculture broodstock are extremely valuable and therefore any technique for induction of maturation and spawning which result in high mortality of broodstock is undesirable (Yano and Wyban,1993). Real breakthrough will only be achieved when it is no longer necessary to use ESA to obtain predictable induced reproduction in captive penaeids which is a long-term goal in shrimp mariculture (Quackenbush,1991).

Out of 40 existing hatcheries in India, only 25 are functional, mainly due to the non-availability of broodstock. The total quantity of seed produced from these hatcheries is around 700 million against a demand of 2000 million and the situation will further aggravate by the end of the eighth five year plan terminating in the year 1997, when the requirement will be around 4000 million (MPEDA,1994). For this, adequate supply of broodstock will be required. Therefore the control of ovarian maturation and spawning of shrimps in captivity is very important as it will be difficult to get from wild at reasonable

price. To achieve this task of production of quality seed more hatcheries are to be established and more simple methods of seed production involving improved broodstock development with predictable peaks of maturation are evolved. Endocrine manipulation without ESA will open up new opportunities for marine shrimp seed production and farming.

Nagabhushanam *et al.*(1982) have appealed to the scientists that the role of steroid hormone on shrimp physiology should be studied in detail in order to achieve artificial induction of gonadal maturation and spawning which might emerge as a more reliable technology than the currently known ESA. Quackenbush (1991) expects that since steroid hormones are present in the reproductive organs and they are proved to be ovarian maturation stimulators in many crustacean species, they can be used for endocrinological manipulation to achieve a breakthrough in the regulation of shrimp reproduction.

In the 1970 s and 1980 s the shrimp reproductive biologists have paid attention for standardizing the method of ESA; but now the time has come for diverting the efforts to develop a new method of broodstock maturation and spawning through administration of exogenous hormones and to replace the ESA technique. With the provision of good environmental conditions and nutrition such a method could produce high quality offspring and be an accepted technique for induced maturation in penaeids, as hypophysation is in the case of carps.

In an attempt to test the claims that steroid hormone treatment can hasten ovarian development and spawning in penaeids, progesterone injection in *P.indicus* was done at three levels of concentrations. As vitamin E is also reported as an   ovarian maturation inducer in shrimp,   it was also included as   one of the test material in the present experiment.

II REVIEW OF LITERATURE

2.1 Reproductive biology of penaeid shrimp

2.1.1 Sexuality and sexual organs in penaeid shrimps.

The penaeids are sexually dimorphic and sexes may be distinguished by the copulatory structures (Forster,1951; Tuma,1967). The male has two pairs of modified abdominal appendages on the first and second abdominal segments (petasma and appendix masculina) that deliver spermatophores into the female's external receptacles (thelycum) located between the base of the fifth walking legs. The thelycum may be open or closed depending on the presence or absence of enclosing plates. The males are generally smaller than the females in size (Muthu,1983). Hall (1962) found that the genitalia develop before the gonads ripen. Thampy and John (1972) suggested the androgenic control of genitalia differentiation in males and Adiyodi and Adiyodi (1970) suggested possibility of an ovarian hormone in females. Internal organs of the male reproductive system include a paired testis, vas deferens and terminal ampoules for spermatophore storage (Heldt, 1938; King,1948; Champion, 1987). The internal female reproductive system includes paired (but partially fused) ovaries that extend from the mid thorax to the posterior end of the abdomen and oviducts terminating adjacent to a single thelycum (Heldt,1938; Hudinaga,1942; King,1948; Cummings,1961; Subrahmanyam, 1965). Ovaries increase in volume and change colour as eggs increase in diameter and accumulate yolk. The external appearance of the ovary was described by King (1948),

Cummings (1961), Subrahmanyam (1965), Tuma (1967), Rao (1968), Villaluz *et al.*(1969), Primavera (1980), Motoh (1981), Crocos and Kerr (1983), Yano (1985) and Tan-Fermin and Pudadera (1989) for various species.

2.1.2 Mating behaviour in penaeids.

Mating occurs as the sexes migrate to the more saline conditions where vitellogenesis and spawning take place (Hall,1962; George and Rao,1968). In penaeids with closed thelycum mating occurs between hard-shelled males and newly moulted females (Hudinaga, 1942; Perez-Farfente,1969; Primavera,1979; Emmerson,1980; Crocos and Kerr,1983). Since penaeids usually moult at night, mating in most of the species with closed thelycum must also be nocturnal. This has been confirmed for *P.japonicus* (Hudinaga,1942), *P.merguiensis* (Aquacop,1975) and *P.monodon* (Primavera,1979). However, De-Saint Brisson (1985) recorded day-time mating for *P.paulensis*. In penaeids with open thelycum it has been suggested that copulation occurs between hard shelled males and females (Perez-Farfente, 1969). In *P. vannamei*, an open thelycum species mating takes place around sunset (Yano *et al.*,1988a). Mating behaviour has been described for eight penaeids: *P.japonicus* (Hudinaga, 1942), *P.stylirostris* (Aquacop,1977b), *P. monodon* (Primavera,1979), *P.paulensis* (De Saint- Brisson,1985), *P. brasiliensis* (Brisson,1986), *P.vannamei* (Yano *et al.*,1988a),*P. semisulcatus* (Browdy,1989) and for *P.schmitti* (Bueno,1990).

As the time for mating of the females of closed thelycum species is very short, it would be advantageous for males and females to be attracted to one another around the time the female moults. Hudinaga (1942) reported that female *P.japonicus* was followed around by males before moulting. The stimulus for courtship behaviour in *Penaeus* spp. is believed to be a sex pheromone secreted in the females' urine and received by the males (Young,1959; Bauchan and Fontaine,1984). Primavera (1985) has suggested that the female penaeids release pheromone to attract males, but presented no evidence to support this claim. Takayanagi *et al.*(1986a) found maturation of female shrimp *Paratya compressa* to be dependent on the presence or absence of males, indicating some sort of pheromone being produced by the males which influence the ovarian maturation. The influence of two separate pheromones, chasing stimulating pheromone (CSP) and mating stimulating pheromone (MSP) has also been discussed by Yano *et al.* (1988a) in *P.vannamei*. Such a sex pheromone has also been demonstrated in many decapod crustaceans including *Palaemonetes vulgaris* (Hazlett,1975), *Palaemon paucidens* (Dunham,1978) and in *Macrobrachium kistensis* (Sarojini *et al.*,1982; Nagabhushanam *et al.*,1989).

2.1.3 Spawning and fertilization.

Mating and spermatophore transfer take place just before spawning in open thelycum penaeids (Heldt,1938), but usually days or weeks before spawning in closed thelycum species (Crocos and Kerr, 1983).However, males will not be present during

spawning in either of the cases (Muthu,1983) and female herself releases sperms from the thelycum (Muthu and Laxminarayana,1982). Spawning behaviour has been described for *P.kerathurus* (Heldt, 1938), *P.japonicus* (Hudinaga,1942), *P.monodon* (Aquacop, 1977a; Primavera,1979; Motoh, 1981) and *P.indicus* (Makinouchi and Primavera,1987) and according to these authors,the spawning is nocturnal.

The penaeids are extremely fecund and may produce from 100,000 to 1,000,000 eggs per spawning in wild (Tuma,1967; Rao,1968; Perez-Farfente,1969). Bray and Lawrence (1992) in their review state that there is positive correlation between female size and number produced and larger species such as *P.monodon* produce higher numbers of eggs than smaller species such as *P.indicus* and *P. semisulcatus*. Muthu (1983) in his review states that the fertilization is external in seawater and occurs as soon as eggs are expelled. Penaeid eggs are shed freely at spawning and are generally thought to be demersal (Garcia and Le Reste, 1981).

2.2 Reproductive biology of *Penaeus indicus*

2.2.1 The size at first maturity.

Subrahmanyam (1963) estimated the size at first maturity for female as 140 mm and for males as 120 mm from Madras coast. Rao (1968) found that the size at first maturity for female from Cochin was 130.2mm. Devi (1987) recorded a minimum size of 125 mm for females at first maturity from Kakinada.

2.2.2 Breeding and spawning season.

Panikkar and Menon (1956) noted two breeding periods in Oct.-Nov. and May-June and it was noticed by George (1962) and George *et al.* (1967) that the species breeds throughout the season with spawning peaks in the same period as above from Cochin waters. In Madras waters the high breeding activity for the species was reported by Subrahmanyam (1963) in Mar.-May to September. Recently Devi (1987) studying the biology of the Indian white shrimp from Kakinada recorded three peak spawning seasons in Jan.-Mar.,Sept.- Oct. and December.

2.2.3 Sex ratio.

Kurup and Rao (1974) reported predominance of females over males during peak breeding season from Ambalappuzha. In Manappad and Punnaikkayal also the females dominated during the spawning season (Manisseri and Manimaran,1981). Devi (1987) also reported the same from Kakinada. But the reverse is the case reported from Cochin coast by George (1962) and George and Rao (1967). Kurup and Rao (1974) and Devi (1986) are of the opinion that the female of the species grows faster than the male and total mortality rate in males is greater than that of females. It was also observed by Devi (1987) that the maximum length attained by females is greater than that of males. Qasim (cited by Devi,1987) attributes, differential growth between the sexes and differences in mortality rate in addition to the spawning migration for the difference in sex ratio, while

Achuthankutty and Parulekar (1986) argue that early attainment of sexual maturity in males is responsible for impairing growth in males.

2.2.4 Fecundity.

Rao (1968) estimated the fecundity at 68,000- 731,000 for specimens measuring 140-200mm total length (TL) size. But it was 110,512- 338,381 for the females of 30-45mm carapace length (CL) (Emmerson,1980). It was 43,330 and 40,230 respectively for ablated and unablated ones from the wild and 23,480 and 26,990 respectively for ablated and non-ablated ones from the pond reared spawners of 91-94 mm TL (Primavera,1982; Primavera *et al.*,1982). Muthu and Laxminarayana (1977) obtained 10,600- 293,000 eggs and Muthu *et al.* (1984) got 45,700- 116,200 eggs from ablated 30-40 mm (CL) *P.indicus*. For 23-30 mm (CL) shrimp the fecundity was 15,480- 80,400 (Makinouchi and Primavera, 1987). Seat and Carlos (1994) got 15,000- 90,000 eggs from 24- 33.5 g *P.indicus*.

2.3 Hormonal regulation of reproduction in shrimp/prawns and other decapod crustaceans

A crustacean gears its reproductive activity in such a way that the liberation of young takes place when conditions are optimal for survival of young. This adaptive synchrony is part of the individuals genetic endowment, which expresses itself via nervous, endocrine and neuroendocrine channels. Various extrinsic cues, such as changes in day length, availability of food, fluctuations in temperature and proximity of prospective mating partners, are noted by the sensory receptors, the resulting afferent

nervous impulses converge on the central nervous system (CNS), which in turn sends directional messages to the concerned organs, thereby eliciting specific response such as promotion or suppression of gametogenesis, uptake of vitellogenin, or sexual receptivity (Adiyodi,1985).

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

Although many observations have been made on the inhibition of reproductive maturation by eyestalk hormone (s), recent research has focused mostly on brain, thoracic ganglion (TG), ovary and mandibular organ (MO) and their functions which are closely related with the release of gonad stimulatory factors or hormone (s) (Yano, 1992a). As with ovarian maturation, it has long been suspected that vitellogenesis in crustaceans is controlled by two antagonistic hormones; in penaeid shrimp GIH is secreted from the

XO-SG complex and inhibits vitellogenesis and GSH is secreted from the TG and brain

and stimulates vitellogenesis (Yano,1992a).

2.3.1 Target gland control.

Dall *et al.*(1990) proposes a target gland control of reproduction in crustaceans. This

target gland control is via GIH and its reduction, either naturally or by ESA, permits the

target gland to function fully. Thus removal of GIH inhibition on the gonads permits them

to develop to maturity. This system is identical to the optic gland of cephalopod molluscs

and corpora allata (CA) of insects (Adiyodi and Adiyodi,1970). But this system is

unusual in that target gland hormones in other animal groups are mostly stimulatory (for

example, vertebrate hypothalamic hormones and insect brain hormone).

2.3.2 Gonadal maturation.

There is still much speculation and divergence of opinion about the process of

gonad maturation and the model of Adiyodi and Adiyodi (1970) is still current

(Adiyodi,1985). This scheme proposes that the actions of moult inhibiting hormone (MIH)

and GIH are antagonistic and also that there is a GSH, produced by the brain and the TG.

Moulting occurs when the titres of MIH and GSH are low and those of GIH and moulting

hormone (s) MH are high; gonad maturation occurs in the converse situation. This model

applies primarily to the females, but there is evidence that in male crabs, GIH acts via the

androgenic gland (AG) by inhibiting its secretion; in its absence maturation takes place

(Payen *et al.*, 1971). In species where moulting and ovarian development alternate, MIH

and GIH must act antagonistically (Dall *et al.*,1990). Moulting and reproduction are generally held to be antagonistic events in malacostracan crustaceans since both require large amounts of energy and are mechanically incompatible (Anilkumar and Adiyodi, 1981; Quackenbush and Herrnkind,1981; Chang,1984,1992).

During the reproductive period the mature crustacean ovary may weigh as much as 10% of the total body weight (Quackenbush and Herrnkind,1981,1983) and during the secondary vitellogenesis the decapod ovary usually increases in weight by 300 % or more depending on the species (Adiyodi,1985; Quackenbush,1986).

2.3.3 Inhibitory factors of gonad maturation.

2.3.3.1 Gonad inhibiting hormone (GIH).

In decapods, it is well known that the removal of eyestalk (ES) induces ovarian activity (Adiyodi and Adiyodi,1970). The existence of a gonad inhibiting principle in eyestalk of decapod crustacean was first demonstrated about 50 years ago by Panouse (1943) with his observation of accelerated ovarian growth in eyestalk ablated female shrimp, *Palaemon serratus*. The neuroendocrine complex produces an inhibitory hormone; when this is removed precocious gonadal development ensues. This was later confirmed in many decapod crustaceans (Stephens,1952 in *Cambarus*, Brown and Jones,1949 in *Uca*, Carlisle,1953 in *Lysmata*, De'meusy and Veillet,1952 in *Carcinus*) including many palaemonids and penaeids (Table 1). Panouse (1944,1946) further found

that the removal of the SG alone leads to some increase in size of ovary,but not nearly so great an increase as after eyestalk ablation (ESA). The effect of ablation of ES or removal of SG on the ovary can be prevented entirely (Panouse,1944,1946; Carlisle, 1953) or partially (Brown and Jones,1949; Rangneker and Deshmukh,1968) by implanting SG tissue into the operated animals. Injection of extracts of whole ES, or SG alone or the ganglionic XO (GXO) alone, prevents any increase in ovarian weight after ES removal (Carlisle,1953), whereas injection of extracts of other fractions has no such effect nor has oral administration of SG after ESA (Knowles and Carlisle,1956). In intact animal the normal increase in ovarian size which precedes the breeding season may be inhibited by injection of extracts of whole ES or SG or medulla terminalis GXO-MTGX- (Carlisle,1953). Knowles and Carlisle (1956) took these results as evidence for existence of an ovary inhibiting hormone (OIH). ESA according to them removes an inhibitor which is preventing ovarian growth, thus leading to rapid uninhibited proliferation of the ovarian tissue which may increase several fold in a month. Conversely, injection of ES Extract (ESE) supplies the inhibitor which keeps the ovary in check.

Though Carlisle (1954) proposed specificity of GIH, Adiyodi and Adiyodi (1970), quoting Otsu (1963), Carlisle (1953), Payen *et al.*(1967) and Juchault and Legrand (1967), argue that OIH and TIH are not different from one another and termed the inhibitory hormone in both sexes as GIH. Quackenbush (1991) agrees to this argument and Chang (1992) finds little reason to suspect that OIH and vitellogenesis inhibiting hormone (VIH)

mentioned by various workers is different from that of GIH. GIH appears to be present

not only in adults, but in immature stages as well; in *Potamon dehaani*, ES of immature

crabs of both sexes have been stated to contain the hormone (Otsu,1963). When

vitellogenesis is already in full swing, ESA does not perceptibly accelerate ovarian growth

suggesting that during this period the synthesis of GIH and/or its release from ES into

general circulation may be very low; the possibility that this may be related to an

optimum is not ruled out (Adiyodi and Adiyodi,1970).

Many workers suggest that GIH is produced by the SG-XO complex in alternation

with a MIH (Laufer and Landau,1991;Yano,1992a). In adult female of several species

ESA results not in moulting, as in juveniles and some adults of some decapods, but in

premature yolk deposition in the ovary, both during the non-breeding season and in certain

species like *Paratelphusa hydrodromous* (Gomez,1965) and *Scylla serrata* (Rangneker

and Deshmukh, 1968) even in prepubertal stages. Adiyodi (1980) suspects that MIH and

GIH represent a single hormone say growth restraining hormone (GRH) that exercises its

influence on two target processes namely, growth and reproduction. Crustacean ES

contains hormone that inhibits moult and reproduction, but the course of events initiated

by ESA varies with species, age of individual, season and other factors (Adiyodi,1985;

Quackenbush,1986; Fingerman, 1987). The production of GIH has been shown to be

seasonal and is responsible for the period of ovarian rest during the non-breeding season

(Adiyodi and Adiyodi,1970; Bomirski and Klek,1974; Klek- Kawinska and Bomirski,1975).

Van Deijuen (cited by Subramoniam and Keller,1993) demonstrated the inhibition of oocyte growth in the shrimp, *Atytephra desmaresti*, by administration of SG extract (SGE) from the lobster, *Homarus americanus*. Quackenbush and Keeley (1987) showed that partially purified ESE from the shrimp *Penaeus vannamei* could inhibit ovarian synthesis of vitellogenin (Vg) of the crab, *Uca pugilator in vitro*, while Eastman-Reks and Fingerman (1984) found inhibition of Vg production in cultured ovaries of this crab. Interestingly, almost all the GIH bioassays thus far found have been heterologous, implying lack of species specificity of this peptide (Subramoniam and Keller,1993).

GIH is thought to exert its effect directly on the ovary and hepatopancreas (HP) *in vivo* since ESE will inhibit protein synthesis by cultured ovaries (Paulus,1984; Paulus and Laufer,1987; Quackenbush,1989; Yano,1992a). The fact that cyclic AMP can mimic this inhibitor suggests its function as an intermediate (Eastman-Reks and Fingerman, 1984). The putative target tissue of the GIH probably respond to ESA by rapid increase in biosynthetic activity of yolk proteins (Quackenbush,1989). Yolk proteins have already been observed in haemolymph of decapod crustaceans undergoing maturation (Byard and Aiken,1984; Derelle *et al.*,1986; Yano,1988; Lee and Puppione,1988).

Primary action of GIH in females apparently occurs during the secondary vitellogenesis, the time when ovary increases dramatically in size due to synthesis and

uptake of yolk proteins produced in either follicle cells or extra ovarian sites (Quackenbush,1991; Chang,1992). But in *P.hydrodromous* there is evidence to show that GIH principally inhibits the primary vitellogenesis in these crabs, however, its effects on secondary vitellogenesis is far from impressive (Kurup and Adiyodi,1980). Alternatively GIH may have non-ovarian target or in fact there may be more than one ES factors which inhibit ovarian growth (Laufer *et al.*,1992).

It was found that water soluble,heat stable ES factor (s) inhibit MO synthesis of methyl farnesoate (MF) and because of a role that MO seems to play in reproduction, that factor (s) may be considered GIH, for it may affect HP,the ovary as well as the MO and is termed mandibular organ-inhibiting hormone (MO-IH) (Laufer *et al.*,1986, 1987a,b; Laufer and Landau,1991) and preliminary reports suggest that ES factor (s) may work through a C GMP intermediate (Tsukimura *et al.*, 1986). Tsukimura *et al.*(1989) also found a ES factor inhibiting MF synthesis in *H.americanus* and *Orconectes virilis*.

GIH, eventhough inhibitory in high titres to ovarian growth, is necessary in declining titres to ensure the proper development of the ovary (Adiyodi and Adiyodi, 1970). GIH is therefore not a GIH in its true sense, but only a gonad restraining hormone (Adiyodi,1980). Anilkumar and Adiyodi (1981) found that removal of GIH by ESA may induce some precocious ovarian development, but such development was not normal.

Kallen and Meusy (1989) have advanced the theory that GIH is similar in structure and not different from crustacean hyperglycemic hormone (CHH). There are indications

that moult inhibition and hyperglycemic activity are associated with the same peptide as demonstrated in the lobster *H.americanus* (Chang *et al.*,1990; Soyez *et al.*,1991) and in the shore crab, *Carcinus maenas* (Webster and Keller,1986). In a similar immunological study in the lobster, indications are that GIH and CHH share common antigens (Subramoniam and Keller,1993).

2.3.3.2 Other inhibitory factors.

The AG which is responsible for the masculanisation of the animal seems to produce a number of compounds including farnesylacetone, a molecule similar in structure to MF (Ferzou *et al.*,1978) and this will inhibit ovarian lipovitellin synthesis *in vitro* (Berreur-Bonnenfant and Lawrence,1984).

It is well known that biogenic amines release peptide neurohormone from neuroendocrine structures in several crustaceans (Fingerman, 1985). Certain biogenic amines (octamine and serotin) inhibited MF synthesis in *Libinia emarginata* (Homola *et al.*,1989). Serotin has been found to induce the release of GIH from isolated ES (Mattson and Spaziani,1985). Tsukimura *et al.* (1986) found that the ESE significantly increased the level of C GMP, but not C AMP in the MO while brain extract and TGE had no effect. These imply the possibility that biogenic amines may stimulate release of GIH from XO-SG complex in crustaceans (Yano,1992a). Landau *et al.* (1989) found that pigment dispersing hormone (PDH) significantly inhibit MO synthesis of MF in *Procambarus clarkii*. Quackenbush and Herrnkind (1983) reported that partially purified

GIH could not be separated from PDH. Thus in some cases the functions of pigment dispersal and gonad inhibition may be performed by the same or similar molecules (Laufer *et al*.,1987b).

2.3.4 Stimulatory factors for control of reproduction.

2.3.4.1 Gonad stimulating hormone (GSH).

A second decapod reproductive neurohormone is found in the brain and TG which acts to stimulate ovarian development in shrimps, crabs and lobsters. The concept of 'bihormonal system' was first proposed by Otsu (1960,1963). Otsu (1963) after working with *Potamon dehaani* and Yano and Wyban (1992) with *P.vannamei* suggested its existence because ESA caused precocious ovarian growth in adult, but not in juveniles. This lead them to reason that not only was the absence of GIH required for ovarian growth, but the presence of a stimulatory hormone was also necessary. Otsu (1963) also observed that implantation of adult TG was effective in triggering maturation of ovary in ESA juveniles. The experiments of Hinsch and Bennett (1979) using *L.emarginata*, Gomez (1965) using *P.hydrodromous* with both brain and TG and Takayanagi *et al*. (1986b) using the shrimp, *Paratya compressa* also proved that GSH from TG has got a role to play in ovarian maturation. Extract of TG of reproductive *Uca pugilator* stimulates ovarian growth in adult crabs (both intact and ablated) while TGE from non-reproductive crabs has no effect on normal crabs and actually inhibited ovarian growth in ablated crabs (Eastman-Reks and Fingerman,1984). Nagabhushanam *et al*. (1988) found that GSH from

brain was more effective than that from the TG in stimulating ovarian growth in *Macrobrachium kistensis*. Yano and Wyban (1992) proposes a GSH-releasing hormone (GSH-RH) from brain of the lobster *H. americanus* which stimulates ovarian maturation in *P.vannamei*. From all these findings, Yano (1992b) speculates that in immature females the ovarian stimulating principle is absent or yet not functioning. Yano (1992b) found that TGE prepared from maturing females is effective in increasing serum Vg in *P.japonicus* and suggested that GSH also stimulates Vg synthesis and/or secretion both into the blood in penaeid shrimp. He further noted that brain extract prepared from maturing females induced Vg secretion in *P.japonicus* suggesting a brain hormone that stimulates release of GSH in penaeid shrimps.

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

The role of GSH appears to be dual in that it promotes oocyte growth and prevents

Y- organ (YO) activity; the latter is accomplished either directly or indirectly by raising

the level of MIH and/or lowering the level of GIH (Adiyodi and Adiyodi,1970).

2.3.4.2 Juvenile hormone (JH).

The role of terpenoid hormones unique to arthropods, and collectively known as

JHs or juvenoids has been established in insect reproduction (Raabe,1982; Laufer *et al.*,

1992). The JH s appear not only in development of insect larval stages, but also in the

regulation of reproduction (Downer and Laufer,1983). In recent years, attention has been

focused on another gland, the MOs as a source of gonad stimulating factor in decapod

crustaceans (Subramoniam and Keller, 1993). Since both the arthropod sub-phyla, the

Insecta and Crustacea, are already known to regulate moulting with identical hormones,

20- hydroxy ecdysone (Karlson,1956; Hampshire and Horn,1966; Laufer *et al.*, 1987b), it

is speculated that the Crustacea might also have a functional JH for development and

reproduction (Laufer *et al.*,1992; Chang *et al.*,1992). This view is supported by

considerable literature. There are reports of insect JH or related compounds having

biological activity in Crustacea and of crustacean tissues having JH activity in insects.

Schneiderman and Gilbert (1958) detected some JH activity in the ES of the Crustacea.

Laufer *et al.* (1987a) identified a sequiterpenoid compound MF in the MO as well as in

the haemolymph of the spider crab. The MF, the immediate precursor of the insect JH III,

has been shown to be present in several decapod crustacean species (Laufer *et al.*, 1986).

In addition, the MO of decapod Crustacea are structurally similar to the CA of insects (Chaudonneret,1956; Le Roux,1968; Byard *et al.*, 1975). If MO is a structural homologue of CA, it is reasoned (Laufer *et al.*,1987b) that it may produce one of the JHs. More interestingly, the activity of MO appears to be controlled by ES, as evidenced by the finding that ESA results in the hypertrophy of this gland (Le Roux, 1983; Laufer *et al.*,1986; Hinsch,1977). MF was isolated and secretory source of MF was detected as MO in the crabs, *L. emarginata*, *Carcinus sapidus*, *C.maenas* and *C.borealis*, the lobster *H. americanus*, the crayfish *Cambarus burtonii* and the shrimps *P.duorarum*, *P.semisulcatus* and *P.vannamei* (Byard *et al.*,1975; Laufer *et al.*,1984, 1987a; Borst *et al.*, 1987). As with JH in insect the indications are that MF has effect upon both larval development and adult reproduction. There are correlation between secretory activity of MO and vitellogenesis in the spider crab *L.emarginata* and high titre of MF was found during peak vitellogenesis (Laufer *et al.*,1987a). MF was also detected in the haemolymph of *Orconectes virilis* and *H.americanus* (Tsukimura *et al.*, 1989). But precise role of MF in vitellogenesis remains unclear (Chang,1992). Farnesoic acid (FA), a precursor to MF is also secreted in large amounts by MO in mud crab such as *S.serrata* (Tobe *et al.*,1989). Wherever the gland was found,its activity always appear to be significantly related to the reproductive state of the organism (Laufer *et al.*,1986, 1987b; Borst *et al.*,1987). The MO of penaeid shrimp is small and difficult to locate in non-reproductive animal (Taketomi and Kawano,1985; Bell and Lightner,1988), but appears to be large and more active in mature specimens (Laufer

and Landau,1991). There are also reports that JH analogue affects crustacean reproduction, in mud crab, *Rhithropanopeus harrissi* (Payen and Costlow,1977) and in spider crab (Hinsch,1981). When Paulus (1984) injected methoprene into *C. maenas* (intact and ablated) the ovaries where apparently enlarged. Taking all these into account, Subramoniam and Keller (1993) propose that MF is the crustacean juvenoid probably involved in the stimulation of vitellogenesis and FA may be a pre-hormone which could undergo conversion to MF or even JH III in the target tissues.

Landau *et al.* (1989) noticed that red pigment concentrating hormone (RPCH) significantly stimulates the rate of uninhibited synthesis of MF by the MO of the crayfish, *P.clarkii*. Experiments of Laufer and Landau (1991) also indicated that RPCH has MO stimulating activity in *P. clarkii* and *Libinia emarginata*.

2.3.4.3 Steroid hormones.

Steroid hormones have been localized by several methods in many crustacean tissues (Skinner,1985; Fingerman,1987). Steroid hormones other than the ecdysone have been found in crustacean eggs, ovarian tissue and the MO (Adiyodi,1985; Couch and Hagino,1983). The location of these steroid hormones, progesterone and estradiol suggests that they may have a role in regulation of reproduction in crustaceans (Fairs *et al.*,1989,1990; Quackenbush,1991; Quinitio *et al.*,1991; Young *et al.*,1992).

2.3.4.3.1 Ecdysteroids (ECD).

The MH, ecdysone is known to play a role in insect reproduction and therefore may act in a similar fashion in crustaceans (Laufer and Landau,1991; Laufer *et al.*,1992). Crustaceans resemble insects in that MH is not required for the maintenance of the gonad once puberty is attained (Adiyodi,1969). YO seems to play a role in the endocrine control of gonadal function, for its ablation in either sex in crabs before the onset of sexual maturity leads to a considerable retardation of gametogenesis and degenerative changes in the gonads (Echalier, 1954; Arvy *et al.*,1956). Though observations of many workers indicate that MH is not essential for reproduction, but only high titre of MIH (Adiyodi and Adiyodi,1970), extirpation of YO from the breeding and juvenile females results in an acceleration of vitellogenesis in *C. maenas* (De`meusy,1963).

There is now a growing body of evidence to suggest that in insects and crustaceans, ECD which are primarily MH (Adiyodi and Adiyodi,1970) are also there in adult life to stimulate the ovarian growth (Adiyodi,1980). Prepubertal growth and development of gonads appear to be part of the normal genetically determined growth process and ECD may have a role in post pubertal development (Adiyodi,1985). The MH may mediate several different aspects of crustacean reproduction and development. There are reports that ECD may be involved in vitellogenesis since it was isolated from the ovaries, in relatively high concentration (Lachaise *et al.*,1981; Chaix and De Reggi,1982; Adiyodi and Subramoniam, 1983). Lachaise and Hoffman (1977) were successful in detecting

several ECD, especially ecdysone in ovaries of crab, *C.maenas* whose titre in ovary registered a gradual increase with the progress of vitellogenesis, with peak levels detected on termination of the process. Experiments have shown that YO removal can result in either stimulation or inhibitory effect on vitellogenesis depending on species, age and stage in the moult stage and reproductive cycle (Chang,1992).

Thus, indications are that ECD also deserves to be classed as 'reproductive' in arthropods (Adiyodi,1980). Detection of ECD in vitellogenic ovary of a number of malacostracans places ECD on par with vertebral gonadal steroid although evidence for their gonadal synthesis is fragmentary (Adiyodi,1985). In crustacean oogenesis, the role of ECD, which is primarily that of MHs in arthropods raises the question of the relative importance of these hormones in moult and reproduction. Because of the untimely initiation and acceleration of proecdysis caused by exogenous ECD,coupled with its inability to accelerate vitellogenesis, it seems reasonable to suggest that ECD functions primarily as MHs in crustaceans and the effect that it may have on reproduction, may be indirect or secondary (Adiyodi,1978). Recent discovery of the presence of ECD in crustacean ovary suggests that the crustacean gonads may secrete or accumulate active hormone principle (Adiyodi,1985; Chang,1991; Young *et al.*,1993).

2.3.4.3.2 Other steroids.

Evidence is accumulating none the less, from scattered works suggesting that the crustacean ovary might play a role in the biosynthesis of steroid hormone (s). Estrogen

was detected in the ovaries of *Panulirus argus* and freshly spawned eggs of the lobster,

H. americanus (Donahue,1940,1948,1952,1957). Lisk (1961) confirmed this estrogenic

compound to be 17-b estradiol. Subsequently, Teshima and Kanazawa (1971a) have found

that the ovaries of *Portunus triturberculatus* posses the enzymes involved in the

conversion of progesterone to 17a-hydroxyprogesterone, testosterone and

deoxycorticosterone. Kanazawa and Teshima (1971) detected progesterone and

testosterone in the ovaries of *Panulirus japonicus*. Estrogen was detected in the ovaries of

the shrimp, *Parapenaeus fissurus* (Jeng *et al.*,1987), *Nephrops norvegicus* and

P.monodon (Fairs *et al.*,1989;1990) and *Pandalus kessleri* (Quinitio *et al.*,1991). A

number of steroids including testosterone, progesterone and pregnenolone have been

identified in the gonads and serum of the crayfish *Astacus leptodactylus* and the lobster

H.americanus (Burns *et al.*,1984; Ollivier *et al.*,1986) and the shrimps *N.norvegicus*,

P.monodon and *Pandalus kessleri* (Fairs *et al.*,1989; Young *et al.*, 1992; Quinitio *et al.*,

1991) and the crab *C.maenas* (Hazel,1986).Couch *et al.* (1987) detected significant levels

of estradiol and progesterone in the MO of *H.americanus*. A molecule very similar to

human chorionic gonadotropin (HCG) was identified in *Palaemon serratus* with the HCG

antibody (Toullec and Wormhoudt,1987).

2.3.4.4 Other factors

Tensen *et al.*(1989) found a stimulatory effect on oocyte growth of the shrimp,

Palaemonetes varians by a peptide of *H.americanus* which is similar to that of CHH; with

regard to the possible existence of such an ovary stimulating hormone in the SG, it will be interesting to know whether this peptide works antagonistically to GIH or synergestically with the putative GSH (Subramoniam and Keller, 1993).

From the investigations of Richardson et al.(1991) on the effect of 5-hydroxy tryptamine on ovary development in the fiddler crab, Uca pugilator, Subramoniam and Keller (1993) speculate that this biogenic amine releases the gonad stimulating neurohormone from brain/TG.

The octomine and serotin not only affect the mating behaviour of lobster, H.americanus (Beltz,1988), an external manifestation of reproductive activity, but also affect MH production which may be an internal manifestation of gonad maturation. According to Laufer and Landau (1991), the same may play a role in the shrimp reproduction.

The interesting possibility of the involvement of prostaglandins in the penaeid shrimp reproduction has been suggested by Middleditch et al.(1979) and supported by D'Croz et al.(1988).

2.3.4.5 External factors influencing hormonal control of reproduction.

Findings on penaeid shrimp ovary maturation by many indicate that the effect of GSH and GIH on induction and inhibition of ovarian maturation or vitellogenesis, is influenced by water temperature and photoperiod. It is possible that release of GSH and

GIH from XO- SG complex or brain/TG may be induced directly or indirectly by water temperature and light stimuli via respectively the antennule (Barber,1961) and eye (Waterman,1961) in female penaeid shrimp (Yano,1992a). The observations of Stephens (1952), Drach (1955), Weitzman (1964) and Cheung (1969) indicate that gonad maturation and therefore possibly GIH secretion like secretion of MIH (Passano,1960; Aiken,1969) may be under the influence of such factors as the season, photoperiod, nutrition, salinity and other factors in the extended environment, acting through sensory receptors. As any visual stimulus which influences the secretion of GIH and MIH, probably acts through eyes, it is interesting to speculate a possible adaptive significance for the location of X-organ close to the eye (Cheung,1969). Aiken (1969) found that ovary maturation required both temperature and photoperiod in proper proportion over a long period in *Orconectes virilis* and these effects are atleast partly channeled through neuroendocrine system.

In the crab *Gecarcinus lateralis* the response of ovaries to ESA is season dependent and the process accelerated by the operation being whatever event that would normally take place next in the unoperated individual (Weitzman,1964). Aoto and Nishida (1956) also reported such a season dependent premature ovary development in functional female of *Pandalus gracilis*. In the crab *Menippe mercenaria* not only is there a similar season dependent alternate dominance of reproductive moulting, but also a transitional period between these two peaks during which ESA may result in either

moulting or reproduction (Cheung,1969). Seasonal dependence of ovarian response may perhaps be much more widespread in higher crustaceans than is currently appreciated; survival value of such a mechanism which must ensure that the energy expended in the reproduction is not wasted, need hardly be emphasized (Adiyodi and Adiyodi,1970).

Lowe (1961) found that in *Cambarellus shufeldti* complete darkness stimulates ovary maturation only in summer and not in autumn; photoperiod may influence the timing of moulting and ovary development by acting on the growth and resorption of HP and ovary in *Orconectes nais* (Rice and Armitage,1974). In *Pachygrapsus marmoratus* an increase in temperature will stimulate ovary growth, but in the absence of ES the effect is more pronounced (Pradeille-Rouquette, 1975). The effect of rainfall on ovary maturation is striking as in *Paratelphusa hydrodromous* (Adiyodi,1968; Anilkumar,1980) and *Metepenaeus macleayi* (Ruello,1973).

2.4 Induced maturation in prawns/shrimps

Securing of ripe spawner from wild is costly and uncertain. This has generated interest in the induced maturation of penaeid shrimps under controlled conditions. Development and management of shrimp broodstock is now an integral part of the hatchery (Muthu and Laxminarayana,1982; Muthu,1983). Male penaeids generally mature in captivity so that induced maturation mainly concerns females and so studies on reproduction have predominantly focused on female maturation (Primavera,1985,1988).

There are three basic approaches employed singly or in combination to induce ovarian maturation in penaeids- endocrine, environmental and nutritional (Primavera,1985). Several studies have demonstrated that successful ovarian maturation is influenced by environmental conditions as well as the hormonal and nutritional status of broodstock (Teshima *et al.*,1989).

2.4.1 Environmental control.

For development of gonads and successful spawning, favourable environmental conditions are necessary. It is apparent from vast literature available on environmental monitoring of reproduction that salinity, light, temperature and pH are of paramount importance for successful reproduction of Crustacea (Bouchon,1991), including penaeids (Lumare,1990). The reproduction in crustaceans can be discussed in terms of physiological and behavioural adaptation of individuals to their environment and therefore seasonal reproduction may be supposed to be the result of interaction between physiology and external cycles in the environment. Crustacean breeding may synchronize with a combination of environmental factors, the relative importance of which may vary in different species and in different environments (Bouchon *et al.*,1992).

2.4.1.1 Light.

The deeper offshore waters where adult shrimps breed is characterized by reduced light intensity and greater penetration of blue and green light compared to other wavelengths (Jerlov,1970; Muthu,1983). Though full maturity was not obtained under

blue, natural light and red light, unablated *P.monodon* under blue and natural light have shown more advanced maturation stage than those exposed to red light and maturation and spawning were obtained from ablated female exposed to natural light (Alava,1979). Unablated *P.duorarum* did not mature in tanks provided with blue, green, and white light (Caillouet,1972). Similarly unablated *P.monodon* attained only partial maturation under blue and natural light but not under red light (Pudadera and Primavera,1981). However, unablated *P.indicus* kept under dim green light and blue light showed improved condition and spawning activity (Emmerson *et al*.,1983). Primavera (1985) obtained maturation in unablated *P.monodon* subjected to different wavelengths in the tank, green light giving best results followed by natural light. Green light combined with ESA induced maturation in wild immature *P.monodon* (Hillier, 1984). Maturation tanks can depend on natural light under a roofed structure or artificial light inside a completely enclosed building with walls and ceiling (Primavera,1988).

Reduced light levels led to fast maturation in non-ablated and ablated *P.monodon* (Emmerson,1983; Hillier,1984). According to Primavera (1985) covered tanks may reduce disturbance and thick covers may also reduce light intensity in maturation tanks. Wyban *et al*.(1987) suggested that reduced light intensity can increase natural spawning and nauplii production for *P.vannamei*. CMFRI (1994) obtained maturation and spawning of *P.indicus* and *P.semisulcatus* by manipulating the light regime to a 5 % level. In almost all the maturation studies the pools were either kept inside a room with weak artificial light or

the tanks had covers that reduced the light intensity to 10-40% of the natural light (Muthu,1983). Brown *et al.*(1979) observed that *P.setiferus* when kept in circular tank painted white on inside sustained injuries by dashing against the wall, but quietened down when the walls were painted black. Emmerson (1980) working with ESA in *P.indicus* noticed that by painting the broodstock pools black on the inner side, the maturation process would be accelerated and the number of spawning and eggs produced by the female and the hatch rate increased. On the other hand, excellent reproduction results were achieved with *P. semisulcatus* by Browdy and Samocha (1985) using black walled tanks with white bottom at low intensity of light. Although early success was seen in unablated *P.japonicus* at high light intensity (Laubier- Bonichon and Laubier,1979; Caubier *et al.*,1979), the tendency in recent years has been to greatly reduce light intensity for shrimp maturation (Aquacop,1979,1983; Browdy and Samocha, 1985; Emmerson,1980). It was speculated that after acclimatization to a light level, ablated *P.vannamei* as well as other species would reproduce over a broad range of light levels (Bray and Lawrence,1992).

The photoperiod promotes the onset of reproduction in *P.vannamei* (Bouchon,1991). The natural photoperiod has two effects on seasonal reproductive cycle, the longer the photoperiod, the earlier the onset of reproduction and greater the number of reproductive females (Bouchon,1991). Unablated *P.duorarum* failed to mature under varying light-dark combinations including complete darkness (Caillouet,1972). Similarly increasing photoperiod to 19 hrs failed to induce maturation in unablated *P.monodon*

(Beard and Wickins,1980). But both ablated and unablated *P.plebejus* produced more maturation and spawning with day length of 14.5 hrs compared to 12 hrs (Crocos,quoted by Primavera,1985). *P.monodon* broodstock are usually maintained either under a natural photoperiod (Aquacop,1977a; Primavera,1985)or a 12-16 hrs photoperiod (Simon,1982). It is also the same for *P.paulensis* (Marchiori and Boff,1983) and *P.plebejus* (Kelemec and Smith,1980) and shorter periods of 8 hrs for subtropical and tropical species like *P.japonicus* and *P.orientalis* (Arnstein and Beard,1975; Kanazawa,1982). Lumare (1979) obtained a longer latency period in ablated *P.kerathurus* at a photoperiod of 12 hrs compared to those under natural day length (9 hrs). The role of photoperiod in the control of maturation is probably not as critical for species distributed along the equator and therefore not exposed to significant differences in day light as it is for subtropical species (Primavera,1985). Since the experiments of Laubier-Bonichon and Laubier (1979) and Caubier *et al.*(1979) on *P. japonicus* and Lumare (1979) on *P.kerathurus* are inconclusive on the exact role of photoperiod, Muthu (1983) doubts that there is any role photoperiod has to perform in tropical penaeids accustomed to a more or less equal day-night regime throughout the year. But a long photoperiod (16D:8L) stimulated spectacular ovarian development in *Parapenaeopsis stylifera* (Nagabhushanam and Joshi,1986).

2.4.1.2 Temperature.

Caillouet (1972) treated *P.duorarum* with increased temperature, but was unsuccessful in producing maturation and spawning. Aquacop (1979) found that *P.*

monodon matured and spawned throughout the year, if the temperature was above 24^0C.

In reproductively active *P.japonicus*, a rest period was induced by reducing temperature

to below 17.5^0C by Lumare (1981). Non-ablated *P.orientalis* had less ovary weight

compared to ESA specimens, though temperature for the former was 18^0C and for the

latter 11^0C (Liang *et al.*,1983;Liu,1983). In both ablated and unablated *P.esculentus*,

increased maturation was obtained at 26^0C compared to 21^0C (Crocos, cited by

Primavera,1985). Zhang (1990) observed that the ovaries of *P.pencillatus* start to mature

at a temperature of 16^0C and temperatures upto 31^0C were suitable for sexual maturation

without ESA. For *P.stylirostris* temperature of 27- 30^0C was found to be better than lower

temperature range of 19-29^0C for reproductive process (Ottogalli *et al.*,1988). Stability of

temperature is considered important for maturation of some species such as *P.vannamei*

and this was not found to be that important for other species such as *P.stylirostris* and

P.indicus (Bray and Lawrence, 1992). Cripe (1994) achieved more number of maturation

and spawning cycles and viable eggs by manipulating water temperature rather than ES

enucleation in *P.duorarum*. Primavera (1985) comparing temperature regimes maintained

in maturation tanks noticed an upper range of 26-31^0C for most penaeids and a lower one

of 16-28^0C for subtropical species such as *P.japonicus* and *P.orientalis* and that for the

latter group ablated females mature even at the lower temperature limits warranting a

greater need of fine tuning of environmental parameters in the absence of ablation.

Temperature manipulation served as a method for inhibiting or stimulating spawning of both open and closed thelycum penaeids (Laubier-Bonichon and Caubier, 1979; Lumare, 1981; Bray and Lawrence, 1984; Kelemec and Smith,1984; Browdy and Samocha,1985; Robertson *et al.*, 1991). One technique sometimes used, as a mild stimulation to spawning, is to use slightly elevated temperature (Laubier and Laubier, 1976), which may help to overcome stress causing resorption of ovaries rather than spawning or atleast hasten the spawning process (Bray and Lawrence,1992).

2.4.1.3 Combination of temperature and photoperiod.

Both high temperature and long photoperiod were found to bring in and maintain breeding in *P.japonicus* (Laubier-Bonichon,1975,1978; Laubier and Laubier,1976; Caubier *et al.*, 1979). Brown *et al.*(1980) in *P.stylirostris* and Chamberlain and Lawrence (1981a) in *P.stylirostris* and *P.vannamei* achieved successful maturation and spawning in ESA as well as non-ESA animals at high temperature and long photophase. Rodriguez (1981) realized that the temperature and photoperiod together play an essential role in maturation of *P.kerathurus*. Chamberlain and Garvais (1984) increased both temperature and photophase and noted that ESA and non-ESA *P.stylirostris* females begin to mature at 25^0C and spawn at 26^0C. The experiments of Crocos and Kerr (1986) revealed that a combination of raised temperature (26^0C) and increasing day length (14.5 hrs) induces maturation and spawning in non-ESA *P.esculentus*; ablation further enhancing maturation under these and also under conditions unfavourable for non-ESA females.

2.4.1.4 Salinity.

It is well-known that penaeid shrimps migrate in the post larval stage into coastal lakes, lagoons, backwaters and estuaries to spend their juvenile phase and move back to the sea for attainment of maturity and subsequent spawning (Mohamed and Rao,1971). The fact that penaeid shrimps which live in brackishwater migrate to the sea for spawning purpose, suggests that salinity is one of the important factors affecting maturation process (Muthu and Laxminarayana,1982). The circumstantial evidence indicates that most penaeids which are unable to reach suitable saline water do not mature (Tuma,1967; George and Rao,1968). Though Muthu (1983) states that in nature shrimps breed only in the sea, the males may become mature in brackishwaters and females never attain full maturity, many reports of penaeids attaining maturity in brackishwater condition have been published. Thus Dakin (1946) and Morris and Bennett (1952) have shown that *Metapenaeus bennettae* from eastern Australia actually breeds in coastal lakes while De Bruin (1965) has recorded mature females of *M.elegans* inside low saline lagoons of Ceylon. *M.burkenroadi* appears to mature inside a coastal lake (Muthu and Manickam,1973). The marine shrimp *M.dobsonii* like all other penaeid shrimps, spawns in the sea and larvae develop in the seawater. Presence of mature females of *M.dobsonii* and their breeding in brackishwater was reported by Rao and Kathirvel (1973) and Silas *et al*.(1982). George (1974) recorded females of this species with ripe ovaries from perennial culture fields of Vypeen Island. Even *P.indicus* in the stage III of maturity have been

collected by George (1974) from brackishwater pond during the high saline months.

Krishnamurthy and Ganapathy (1985) reported that sufficiently old *P.indicus* may

develop ovary even in brackishwater. Kathirvel (1985) included *P.japonicus* among the

shrimps attaining maturity in low saline confined waters. Other species found to mature in

confined waters are *P.setiferus* (Johnson and Fielding,1956), *P.monodon* (Villaluz *et al.*,

1969), *M.bennettae* (Racek,1973), *P.merguiensis* (Lichatowich *et al.*,1978;

Gopalakrishnan *et al.*,1987), *P.stylirostris* (Conte *et al.*,1977), *P.kerathurus* (Rodriguez,

1981) and *P.latisulcatus* (Kathirvel and Selvaraj,1987). The salinity of the pond at the time

of collection of maturing shrimps was 12.04-16.5 ppt. *P.japonicus* (Kathirvel and

Selvaraj,1989), and 34.2-45.5 ppt. *P.kerathurus* (Rodriguez,1981), *M.dobsonii* (Silas *et*

al.,1982) and *P.merguiensis* (Gopalakrishnan *et al.*,1987), indicating that maturation of

penaeid shrimps can take place either in brackish, saline or hypersaline waters (Kathirvel

and Selvaraj,1989).

Most maturation systems depend on available seawater with ambient salinity of

24-36 ppt.(Primavera,1985). Although salinities of 28-36 ppt. are recommended for

maturation, published data are lacking (Ogle,1992a). Though it is generally held that in

nature penaeid shrimps mature and breed only in the sea, the reports that some of them can

also mature in brackishwater salinity lead scientists to test the validity of such a claim

under controlled conditions. The range of salinity reported for maturation of *P.setiferus* is

22-44 ppt.(Conte *et al.*,1977; Brown *et al.*,1979; Lawrence *et al.*,1980; Chamberlain,

1988). Ogle (1992b) induced the same species at lower salinities of 20 ppt., 25 ppt., as well as 30 ppt. to mature and spawn. Caillouet (1972) was not able to induce maturation in *P.duorarum* by manipulation of salinity. The salinities reported for maturation of *P. stylirostris* vary between 16 and 38 ppt.(Aquacop,1979; Lawrence *et al.*, 1980; Brown *et al.*,1980; Chamberlain and Lawrence, 1981a,b). Halder (1978,1980) reported attainment of maturity and spawning of viable eggs in ablated *P.monodon* under brackishwater salinity of 25 ppt. Posadas (1986) showed that ablated *P.monodon* can mature and spawn at 15, 25 and 32 ppt., but requires seawater salinity for incubation and hatching of eggs. Openiano (quoted by Primavera, 1985) noticed no significant difference in maturation rates of *P.indicus* kept in the tanks at 22, 32 and 42 ppt., although females that had matured at 32ppt. showed significantly higher fecundity and hatch rates. Maturation and spawning in *P.vannamei* were reported to occur at 20 ppt.(Thompson, cited by Ogle, 1992a), though the larvae had to be transferred to higher salinities for rearing. In a preliminary experiment Ogle (1992a) saw no significant difference for *P.vannamei* spawned in either natural or artificial water at salinities of 20, 25 and 30 ppt.

2.4.1.5 pH.

Best results were obtained when oceanic water at steady pH of 8.2 was made to continuously flow through the maturation pools (Aquacop,1977a,1979). Ablated *P.indicus* females reached early maturation and then resorbed their ovary when the pH of recirculated water was allowed to decline from 8.2 to 7.2 in plastic pools (Muthu *et*

al.,1984); they got successful spawning of viable nauplii only when the pH was maintained at 8.2. When non- ablated *P.indicus* and *P.semisulcatus* were kept in maturation pools at reduced light intensity and at a seawater pH of 8-8.2 successful maturation and spawning were obtained (CMFRI, 1994). A pH range of 7.1-8.2 for *P.indicus* (Emmerson,1980; Primavera *et al*.,1982; Aquacop,1983; Emmerson *et al*., 1983) and 7.8-8.2 for *P.monodon* (Aquacop,1977a,1979; Primavera,1978; Pudadera and Primavera,1981; Ruangpanit *et al*.,1981) were found to be suitable for maturation.

2.4.2 Nutritional factors.

Nutrition is profoundly important for reproduction of *Penaeus* spp. and the success of reproduction closely relates to nutrient ingestion accompanying ovarian development (Bray and Lawrence,1992). Although our knowledge of nutrient requirement of broodstock shrimp is limited (Harrison,1990), nutritional requirements need to be met as the condition of the broodstock will be reflected in the quality of eggs spawned (Lawrence *et al*.,1979; Primavera *et al*.,1979; Aquacop,1983; Xu *et al*.,1994). Penaeid diets are yet to be defined in terms of qualitative and quantitative nutritional requirements. The lack of clearly defined nutritional requirements for reproduction has been a constraint to both development of satisfactory dry feeds for broodstock and production of consistently high quality nauplii. Live or fresh frozen components used are expensive, may deteriorate water quality and may vary in nutritional quality with species, age, maturation state,season and location (Bray *et al*.,1990).

2.4.2.1 Food sources.

Although nutrition is considered one of the most important factors in reproduction, only very little research has been conducted to evaluate the relative suitability of various food items for maturation. Molluscs including mussel, clam, cockle and squid are the most common food sources for penaeid broodstock. Other food items are fresh or fresh frozen marine worms, mysids, shrimps, fish and dried pellets. These items are given alone or in combination (Lumare,1990). The breeders are fed *ad libitum* or according to a daily feeding ration of approximately 3-5 % (pellets) and 10-30 % (fresh or fresh frozen) of body weight. It is given once upto four times a day and daily ration divided accordingly (Primavera,1985). A high protein diet rich in essential amino acid (EAA) and long chain highly unsaturated fatty acid (HUFA) appear to be necessary for maturation of ovaries (Muthu,1983). It is generally accepted that maturation of female shrimp requires a large amount of dietary component of fresh feed (Ogle,1988). A range of natural feed stuffs is used (Muthu and Laxminarayana, 1982; Primavera,1985; Harrison,1990), but choice of diet seems to depend on local availability, preferences and resulting reproductive performance. The diet usually contains squid which when used for *P.vannamei* and *P.stylirostris* (Chamberlain and Lawrence,1981b) and for *P.vannamei* (Chamberlain,1988) produced better results than any other single item diet. The poor results obtained with clam meat in *P.vannamei* and *P.stylirostris* (Chamberlain and Lawrence,1981b) and other molluscs in *P.kerathurus* (Luis and Ponte,1993) contrast sharply with excellent results

obtained using different molluscs to mature *P. japonicus* (Laubier and Laubier,1976; Laubier-Bonichon,1978; Kanazawa,1990), *P.kerathurus* (Lumare,1979), *P.merguiensis* (Beard *et al.*,1977), *P. plebejus* (Kelemec and Smith, 1980) and *P.monodon* (Primavera *et al.*,1979; Beard and Wickins,1980). However, except in the studies by Laubier and Laubier (1976) and Lumare (1979) each of these studies used another food type to supplement the mussel diet. Muthu and Laxminarayana (1982) and Muthu (1983) observed that visceral mass of clam used for feeding broodstock which generally contains developing gonads probably provides a right type of fatty acid, lipoproteins and carotenoids essential for vitellogenesis in maturing shrimp. A similar view is also held by Aquacop (1977a) and Chamberlain and Lawrence (1981b). More frequent occurrence of molluscs and other non-crustaceans during months when *P.monodon* showed high breeding index reflects change in dietary requirement related to gonadal development during the spawning season (Marte,1982). On the basis of EAA and EFA profiles and ovarian profile of *P.monodon*, a combination of squid mantle and green lipped mussel *Perna canaliculus* was recommended by Marsden *et al.*(1992) to provide a good foundation for the fresh component of maturation diet, the former containing concentration of high quality protein and the latter having high levels of total lipids. Luis and Ponte (1993) noticed that polycheate worms are suitable for inducing gonadal growth and spawning in *P.kerathurus*. The importance this was stressed by Brown *et al.*(1979), Middleditch *et al.* (1979), D' Croz *et al.*(1988) and Lytle *et al.*(1990) also.

Diets consisting of one or more fresh/fresh frozen marine animal ingredients, the most common being squid, mussel, clam, shrimp, brine shrimp, polycheate worms and additional being fish, mysids, trocha, krills, cockles,crab and other items, have been reported as broodstock diets (Bray and Lawrence,1992). In addition to fresh marine animal food source, a macroalgae *Enteromorpha* sp. has been introduced as a staple food for *P.stylirostris* (Bray *et al.*,1990) and *P.monodon* (Bray and Lawrence,1988) and its meal added to a prepared diet for *P.indicus* (Emmerson,1980; Emmerson *et al.*,1983). Bray *et al.*(1989) observed that soybean supplementation increased nauplii production in diets containing multiple fresh feed and they generally outperform squid supplement. Browdy *et al.*(1989) provided evidence that an *Artemia* supplement increases reproductive performance in *P.semisulcatus*.

2.4.2.2 Biochemical Composition.

In penaeid shrimps (Teshima and Kanazawa,1983; Castille and Lawrence, 1991; Millamena and Pascual,1990), it is noted that protein, carbohydrate and lipid are mobilized from HP to ovary prior to spawning. The trend in fluctuation of metabolites during ovarian growth of *P.indicus* indicated that in ovary there is characteristic accumulation of protein, lipid, cholesterol and carotenoids, but loss of moisture and nucleic acids (Mohamed and Diwan,1992). In terms of abundance protein is the largest organic constituent of ovary and lipid second. Carbohydrate is present at much lower level than

these two, but in terms of relative change increase in lipid is greater than those of protein and carbohydrate (Castille and Lawrence,1991).

2.4.2.3 Lipids.

Recent studies on nutritional requirement of penaeids have focused on lipids rather than protein which provide energy as well as sterols; phospholipids (PL) and EFA are believed to be key nutritional factors influencing egg hatching and larval survival (Primavera, 1985; Cahu *et al.*,1987; Marsden *et al.*,1992; Xu *et al.*,1994). Lipids in particular are suspected to largely affect the reproduction process and egg quality in *P.duorarum* (Gehring, 1974), *P.japonicus* (Gyary *et al.*,1974; Teshima and Kanazawa, 1983; Teshima *et al.*,1988b, 1989), *P.setiferus* (Lawrence *et al.*, 1979; Middleditch *et al.*,1979,1980), *P.indicus* (Read and Caulton,1980; Galois,1984; Cahu *et al.*,1988), *P.monodon* (Millamena *et al.*,1985; Millamena and Pascual,1990) and *P.stylirostris* (Bray *et al.*,1990). Because of the limited ability of shrimp to synthesize long chain HUFA and the rapid rate of ovarian tissue synthesis due to accelerated rate of ovarian development with ESA, a higher requirement for lipid during breeding of penaeids is suggested, from immediate ingestion. This is due to the limited capacity of the HP to store the lipid (Bray and Lawrence,1988). There are two families of HUFA, EFA n-6 or linoleic family (C18:2 n-6) and n-3 or linolenic family (C18:3 n-3). The HUFA which are particularly important for reproduction of penaeids include arachidonic acid (AA- C20:4 n-6), eichosapentaenoic acid (EPA-C20:5 n-3) and docosahexaenoic acid (DHA-C22:6 n-3) and their presence in

spawned eggs indicate their importance (Primavera, 1985). To understand the logic of using blood worms based upon nutrient matching a sterol analysis of mature shrimp ovary with potential feed is suggested by Middleditch *et al.* (1979,1980). Middleditch *et al.*(1979) suggested that AA present in these worms might be the key HUFA which is the precursor of prostaglandins and since certain prostaglandins have been used extensively as drug for human reproduction (Johnson *et al.*,1983) it is suggested that prostaglandins may play some role in shrimp reproduction as well (D' Croz *et al.*,1988; Lytle *et al.*,1990).

Since studies have shown that high concentration of n-3 HUFA must be there in shrimp maturation diet, Lytle *et al.*(1990) hypothesized that there exists a delicate balance of n-3 and n-6 FA, a view shared with Kinsella (1988) and Millamena (1989). Analytical results suggest that oysters, squid and certain worms are similar to commercial diets which have high absolute concentration of n-3 and the ratio of n-3/n-6 FA is dissimilar from that of bloodworms (Middleditch *et al.*,1980; Lytle and Lytle,1990). The balance of n-3 and n-6 FA (Kinsella,1988; Millamena,1989; Lytle *et al.*,1990) and AA (Middleditch *et al.*,1979,1980; D' Croz *et al.*,1988; Luis and Ponte,1993) have been suggested to be limiting factors. Cahu *et al.*(1986) confirmed that eggs of a female fed a pellet diet only, were lower in AA,EPA and DHA and higher in linoleic acid and had different ratio of FA compared to those getting diets containing part or all fresh mussel. Penaeids have limited ability to elongate and desaturate 18 carbon FA to EPA and DHA (Teshima *et al.*,1989). This together with the observation that fecundity and hatch rate of shrimp depend on the

diet fed to the broodstock suggest the importance of HUFA in the maturation diet of penaeid shrimp (Cahu *et al.*,1994; Xu *et al.*,1994). Middleditch *et al.*(1980) have demonstrated that *P.setiferus* would not produce eggs unless diet contained EPA and DHA and so suggested that long chain HUFA are essential for vitellogenesis of penaeid shrimps. A similar condition was reported in *P.vannamei* (Lytle *et al.*,1990). Through a feeding study, Alava *et al.* (1993a) concluded that *P.japonicus* requires n-3 HUFA and PL for ovarian growth and that PL is responsible for the increase in the ovarian lipid content. The polar lipids in the ovary during ovarian growth are mainly responsible for the increase in concentration of lipid in the ovary of penaeid shrimps (Galois,1984; Millamena and Pascual,1990; Teshima *et al.*,1989; Cahu *et al.*,1994) which is suggestive of significance of phospholipid in the ovarian development of shrimps. Complete gonadal maturation and spawning performance have been obtained in *P.monodon* and *P.indicus* fed diets containing PL and/or n-3 HUFA and results indicate that requirement varies from species to species (Millamena,1989; Cahu *et al.*,1991). Cahu *et al.*(1994) proposed that EPA has some specific role in ovarian development process relating to fecundity whereas DHA has some other role in early embryogenesis in *P. vannamei*. Though cholesterol (or other sterols) required for reproduction has not been defined, dietary source of sterols have been shown to be required for growth in Crustacea implicated as precursor of steroid hormones (Kanazawa *et al.*,1971a,b; Kanazawa and Teshima,1971; Teshima and Kanazawa,1986; Kinsella,1988) and important in oogenesis (Kanazawa *et al.*,1988).

Findings of experiments point to the need for natural food sources particularly those rich in HUFA for penaeid maturation since shrimps are not capable of bio-synthesis of HUFA (Cahu *et al.*,1994) and these should be supplied through the diet of broodstock in adequate amount for proper ovary growth (Primavera,1985).

2.4.2.4 Proteins.

The information provided by Dy-Penaflorida and Millamena (1990) and Mohamed and Diwan (1992) are useful as in the absence of studies on amino acid (aa) requirement, diet development is based on the tissue profile. It has been shown that ovarian protein increases with ovarian development in shrimp (Rankin *et al.*,1989; Dy-Penaflorida and Millamena, 1990; Quinitio *et al.*,1990; Quinitio and Millamena,1992; Chang *et al.*, 1994). As in most crustacean eggs, the eggs of penaeids contain approximately 24 % of protein and 22 % lipid (Pandian,1967;Harrison, 1990). In *P.indicus* ovarian protein level varied from 17-39 % during ovarian growth and mature yolk contained 39.9 % of protein on a dry weight basis (Mohamed and Diwan,1992). Current commercial diets contain 40-60 % protein, but optimum protein level has not been determined and improving the quality of protein by providing a balance of aa profile may enable the levels of dietary protein to be minimized (Marsden *et al.*,1992).

2.4.2.5 Vitamins.

Vitamin and mineral requirements have not been defined although some recent works with breeders suggest that definite requirement for vitamin and minerals established

for larvae be used as a guide (Bray and Lawrence,1992). Vitamin E activity is present in a group of naturally occurring closely related tocopherols among which a-tocopherol is having higher vitamin E activity (He and Lawrence,1993). As a fat soluble antioxidant a major role of vitamin E is to prevent peroxidation of HUFA in cellular and sub cellular membranes. Most of the deficiency signs in fish such as nutritional muscular dystrophy, fatty liver degeneration, anaemia, exudative anathesis, erythrocyte haemolysis, haemorrhage, depigmentation and reduction of fertility are related to peroxidative damage to cellular membranes (NRC,1983). Since HUFA are essential in diets of penaeids (Kanazawa,1985) it is postulated that as a metabolic antioxidant vitamin E will also be important (NRC,1983), and may also be necessary in prevention of peroxidation of HUFA in feed (He and Lawrence,1993). Vitamin E is found to be indispensable in broodstock diets of ayu, carp, rainbow trout and red sea bream (Watanabe,1985).

Some recent works on vitamin E reveal its importance in shrimp reproduction. Castillo *et al.*(1989) noticed that in *P.indicus* stock of a- tocopherol in HP and muscles are depleted during ovarian growth and a-tocopherol is transferred to egg during the process and though a-tocopherol level of eggs decreases during successive spawning, no significant decrease in egg hatchability was observed. It is documented that high vitamin E is required for increased hatch rate (HR) when *P.indicus* spawns multiple times (Cahu and Fakhfakh,1990; Fakhfakh and Cahu,1990). Cahu *et al.*(1991,1993) noted that vitamin E plays very important role in reproduction and it is found to be required for *P.indicus*

broodstock diet which affects the quality of eggs in terms of HR and larval survival.

Fakhfakh *et al.*(1991) clearly demonstrated that in broodstock organs and eggs of *P.indicus* vitamin E acts as a biological antioxidant conversely to HUFA peroxidation and they also suspect some specific role for this vitamin in embryonic development. Though fat soluble vitamins A and E contained in the basic ingredients were sufficient to maintain survival, for significantly better weight gain and ovarian growth, supplementation of both vitamin A and E is necessary (Alava *et al.*, 1993b). In a study Chamberlain (1988) documented significantly lower percentage of normal sperm from shrimp fed on vitamin E deficient diet compared to shrimp fed on diets containing vitamin E. Gyary *et al.* (1976) recorded accumulation of vitamin C during ovarian growth in *Palaemon serratus* and Alava *et al.*(1993c) demonstrated that if *P.japonicus* is subjected to ESA, supplementation of vitamin C is critical for survival, resistance to stress due to ESA and promotion of ovarian growth.

2.4.3 Hormonal manipulation.

2.4.3.1 Eyestalk ablation.

Eversince the observation of Panouse (1943) that ESA leads to ovarian growth in sexually quiescent *Palaemon serratus*, ESA has been used as a tool by several workers to induce maturation and spawning in many species of shrimps and prawns (Table 1).

In the ES of decapod crustaceans a GIH is produced by the NSC of XO and transported to the SG for storage and release, thus ESA destroys XO-SG complex and

Table 1.Experiments on reproduction in shrimps/prawns using ESA.

Species	References
Artemesia longinari	Petriella and Diaz,1987.
Caridina rajadhari	Persis and Sarojini,1985.
Macrobrachium nobilii	Kumari and Pandian,1987.
M.malcomsonii	Murugadass *et al.*,1988.
M. rosenbergii	Amaldoss *et al.*,1991.
M. idella idella	Jayachandran and Jose,1993.
M. equidens	Bijulal,1994.
Palaemon paucidens	Kamaguchi,1971.
P. serratus	Panouse,1943.
Penaeus aztecus	Aquacop,1977a,1979.
P. canaliculatus	Choy,1987.
P. duorarum	Idyll,1971; Caillouet,1972.
P. esculentus	Crocos and Kerr,1986.
P. indicus	Muthu and Laxminarayana,1977,1979;Primavera and Yap,1979; Primavera *et al.*,1979,1982; Emmerson,1980; Makinouchi and Primavera,1987; Galgani *et al.*,1989; Mohamed and Diwan,1991; Seat and Carlos,1994.
P. japonicus	Aquacop,1977a; Lumare,1981; Yano,1984;
P. kerathurus	Lumare,1979; Luis and Ponte,1993.
P. merguiensis	Alikunhi *et al.*,1975; Nurjana and Yang,1976.
P. monodon	Arnstein and Beard,1975;Alikunhi *et al.*,1975; Wear and Santiago,1976; Chen,1977; Santiago,1977; Aquacop, 1977a, 1979, 1982; Muthu and Laxminarayana, 1977, 1979; Halder,1978,1980; Primavera,1978; Primaera *et al.*,1979,1982; Rodriguez,1979; Beard and Wickins,1980; Emmerson,1983;Poernomo and Hamami,1983; Hillier, 1984; Rajyalakshmi *et al.*,1988; Tan-Fermin,1991; Menasveta *et al.*,1993,1994.
P. notialis	Ramos,1985; Trujillo and Primavera,1986.
P. orientalis	Arnstein and Beard,1975.
P. paulensis	Marchiori and Boff,1983.
P. plebejus	Kelemec and Smith,1980,1984.
P. schmitti	Bueno,1990; Nascimento *et al.*,1991.
P. semisulcatus	Browdy and Samocha,1985; Browdy *et al.*,1986.
P. setiferus	Brown *et al.*,1979,1980; Lawrence *et al.*,1980; Ogle,1992b.
P. stylirostris	Aquacop,1979; Chamberlain and Lawrence, 1981a; Ottogalli *et al.*,1988;Bray *et al.*, 1989,1990.
P. vannamei	Aquacop,1979;Chamberlain and Lawrence, 1981a,b; Wyban *et al.*,1987; Chen *et al.*, 1991; Yano and Wyban,1993

leads to either precocious moulting or gonadal maturation depending upon the interactions

of ambient temperature and age of animal (Adiyodi and Adiyodi,1970). Except for a few

studies, endocrine manipulation has been synonymous with ESA, a technique first

performed in the penaeid shrimp, *P.duorarum* by Idyll (1971) and Caillouet (1972) with

far reaching impact on aquaculture (Primavera,1985). Now the process of unilateral ESA

(UESA) is used in almost all the marine shrimp maturation/reproduction facilities in the

world, both research and commercial to stimulate female shrimp to develop mature

ovaries and to spawn (Bray and Lawrence,1992). Some workers including pioneers tried

bilateral ESA (BESA), but the results obtained by them were not encouraging. BESA,

though leads to rapid ovarian development, result in high mortality, inability of females to

spawn, loss of balance, spiral swimming behaviour and other abnormalities (Caillouet,

1972; Aquacop,1975; Arnstein and Beard,1975; Duronslet *et al.*,1975; Santiago,1977;

Muthu and Laxminarayana,1977; Beard and Wickins,1980; Marchiori and Boff,1983;

Mohamed and Diwan,1991) which prompted scientists to abandon this method. The

reports of successful spawning of BESA are those of Alikunhi *et al.* (1975) who stated

that *P. merguiensis* and *P.monodon* spawned after BESA and Rajyalakshmi *et al.* (1988) in

P.monodon, where mortality was very high. Very soon it was realized that UESA

successfully lead to ovarian growth and subsequent spawning (Arnstein and Beard,1975;

Wear and Santiago,1976; Aquacop,1977a; Santiago,1977; Halder, 1978; Muthu and

Laxminarayana,1977;Primavera,1978;Primavera and Yap,1979;Rodriguez,1979; Lumare,

1979; Emmerson,1980). Various factors such as age, food, stress, salinity, pH, temperature

and light seem to influence the sequence of events that follow ESA (Muthu and

Laxminarayana,1982). Penaeids in captivity may be divided into a difficult-to-breed group

that requires ablation to mature, like *P.aztecus*, *P.duorarum*, *P.monodon* and *P.orientalis*

and those that mature without ablation like *P. californiensis*, *P.indicus*, *P.japonicus* and *P.*

merguiensis (Primavera,1985).Unablated controls did not mature or had a lower

maturation rate than ablated *P.aztecus* (Aquacop,1975), *P.esculentus* (Crocos and

Kerr,1986), *P.monodon* (Aquacop, 1977a; Santiago,1977; Hillier,1984) *P.plebejus*

(Kelemec and Smith,1980), *P. canaliculatus* (Choy,1987) and *Artemesia longinaris*

(Petriella and Diaz,1987). Ablation increased maturation and spawning in *P.setiferus* and

P.stylirostris (Brown *et al.*,1979; Lawrence *et al.*,1980; Chamberlain and

Lawrence,1981a), *P.indicus* (Emmerson,1980), *P.kerathurus* (Lumare,1979), *P.japonicus*

(Lumare,1981),*P.monodon* (Arnstein and Beard,1975; Beard and Wickins,1980;

Hillier,1984) and *P. orientalis* (Arnstein and Beard,1975) compared to lower number of

spawning in unablated *P.japonicus* (Laubier and Laubier,1976) and *P. merguiensis* (Beard

et al.,1977; Crocos and Kerr,1983). Aquacop (1979) reported that UESA is required for

maturation and spawning of *P. aztecus* and *P.monodon*, and leads to accelerated ovarian

development in *P. vannamei* and *P.stylirostris*, although ESA is not essential for

maturation in the latter two species. In *Parapenaeopsis hardwickii* and *P.monodon*

according to Kulkarni and Nagabhushanam (1980) and Emmerson (1983) the first to

spawn is the ablated ones, probably due to the lower blood titre of OIH. Unablated

P.indicus and *P.monodon* took longer time for ovarian development

(Emmerson,1980,1983). It was reported that UESA is the method of choice for induced

ovarian development in *P. vannamei* whose size is critical for the successful ovarian

development (Wyban *et al.*,1987; Chen *et al.*,1991). Bueno (1990) opines that year round

production of *P.schmitti* can be achieved through UESA. Mohamed and Diwan (1991)

hypothesized that partial removal of inhibitory hormone IH, through UESA favours

ovarian growth, whereas total removal of IH, through BESA result in moulting of

P.indicus. ESA increases total egg production in captivity by producing more frequent

spawning but no larger spawn (Browdy and Samocha,1985). Usually among somewhat

larger individuals spawning is seen in unablated females, but frequency of spawning is

higher in ESA animals (Emmerson, 1980; Browdy and Samocha, 1985; Browdy *et*

al.,1986). Reduced fecundity was observed for captive generation of *P.stylirostris*

(Magarelli,Jr.,1981), *P.semisulcatus* (Browdy *et al.*,1986) and *P.canaliculatus*

(Choy,1987). Higher mortality of ESA female is often, but not always reported (Caillouet,

1972; Alikunhi *et al.*,1975; Aquacop,1977a; Santiago,1977; Primavera *et al.*,1982;

Lumare, 1979; Vincente *et al.*,1979; Chamberlain and Lawrence,1981a; Poernomo and

Hamami,1983; Makinouchi and Primavera,1987; Petriella and Diaz,1987; Rajyalakshmi *et*

al.,1988; Bueno, 1990). ESA deteriorates condition and produces lower egg quality and

HR (Emmerson,1980; Lin and Ting,1986; Choy,1987; Yano and Wyban,1993). While

many of the undesirable characters sometimes observed in captive reproduction do not appear related to ESA, there is some evidence that ESA causes abnormalities which could be expected to reduce quality of offspring (Primavera,1985; Bray and Lawrence,1992), but this view is opposed by Tan- Fermin (1991) from the findings in *P.monodon* that ESA does not produce any abnormal egg.

The latency period from ablation to onset of maturation and subsequent spawning is reduced by 3 to 21 days depending on the age and source of broodstock, stage of moult cycle and other factors at the time of ablation (Primavera,1985). The interval between consecutive spawnings is reduced to only 3-15 days in ablated females compared to a minimum of 10 days upto 2.7 months in unablated and wild females. A decline in fecundity, HR and egg viability has been observed in single spawning with successive moult cycle in *P.monodon* (Beard and Wickins,1980) and in *P.indicus* (Emmerson,1980; Primavera *et al.*, 1982) and an increase in proportion of partially developed ovaries and partial spawning in successive spawning in *P.kerathurus* (Lumare, 1979). Given this reproductive decline, *P. monodon* has to be replaced 6-8 months (Simon,1982; Primavera,1985), *P.indicus* 7 months (Emmerson,1980) and *P.schmitti* 3 months (Bueno,1990) after ESA. But findings of Wyban *et al.*(1987) contradict this and they claim that ESA does not affect the useful reproductive life of *P.vannamei*. Ablated females were found to remature and repeatedly spawn after ablation (Primavera,1978; Aquacop,1979; Lumare,1979; Primavera *et al.*,1979; Yano,1984).

There are a number of methods to perform ESA. Cutting the ES near the base with a pair of scissors (Arnstein and Beard,1975; Lumare,1979), scissor cutting and soldering with pencil type iron solder (Caillouet, 1972), electro-cauterization (Muthu and Laxminarayana, 1979), pinching the eye (Aquacop,1977a), incision of eyeball, squeezing the contents and crushing the ES (Primavera,1978), squeezing the eyeball contents outwards (Rodriguez,1979), incision of eyeball followed by enucleation of contents (Kelemec and Smith,1980), ligation of ES (Makinouchi and Primavera,1987), removing the ES by a lancelet (Chen,1979) and ESA with a hot surgical clamp (Duronslet *et al.*,1975) are some of the methods used for getting rid of ES containing XO-SG complex which secretes, stores and releases GIH. The methods of Arnstein and Beard (1975) and Rodriguez (1979) are not suitable for delicate species like *P.indicus* (Muthu and Laxminarayana,1982). Makinouchi and Primavera (1987) compared different techniques of ESA and found high survival and HR for female *P.indicus* using either cauterization or ligation which is comparable to that of unablated ones, whereas survival and HR were significantly lower for specimens ablated by eye pinching. Muthu and Laxminarayana (1979) and Browdy and Samocha (1985) also got high survival for penaeids ablated by cautery apparatus.

Primavera (1985) advises to carry out ablation during the intermoult period for maturation to follow in less than a week. Ablation in premoult leads to moulting and a longer latency period of 2-4 weeks for *P.monodon* (Aquacop,1979; Primavera *et al.*,1979).

On other hand ablation during post moult lead to mortality because of added stress on the female and extensive loss of haemolymph (Aquacop,1977a).

2.4.3.2 Exogenous agents.

Kulkarni *et al.*(1979) observed stimulation of oogenesis in *Parapenaeopsis hardwickii* by the injection of progesterone at 10 ug on alternate days for 10-20 days. Nagabhushanam *et al.*(1980,1982) induced spawning in *P.stylifera* by administering 17-a Hydroxyprogesterone (17-a HP- 50 ug/shrimp). In the same species greater GSI and egg diameter were observed in females injected with progesterone compared to ethanol treated and un-injected controls (Joshi,1980). Sarojini and Gyananath (1984) detected proliferation of oocytes and deposition of yolk and subsequent vitellogenesis in progesterone treated female *Macrobrachium lamerrii*. More advanced stages of ovarian development was obtained in the progesterone treated females (0.1ug/g) and 2 of them spawned nocturnally after 30 and 31 days of injection (Yano,1985). Administration of progesterone into immature *M.kistensis* accelerated oogenesis (Sarojini *et al.*,1985). Yano (1987) recorded increased level of Vg production in 17-a HP treated (0.01 ug/g) *P.japonicus*. Nagabhushanam *et al.* (1987) injected *Parapenaeopsis stylifera* with progesterone to get accelerated oogenesis and 17-a HP to get spawning even at a low temperature of 20^0 C, at which the species does not spawn naturally. Though external viewing and GSI values after 20 days showed no significant ovarian growth when compared to animals in the wild, histological structure demonstrated distinct changes in

the type and size of oocytes following progesterone treatment indicating stimulation of oogenesis by hormone treatment in *P.merguiensis* (Chan and Lim,1988). Recently, evidence has been produced to show that 17-a HP stimulated synthesis and/or release Vg into blood of *M.lanchesteri* (George and Khoo,1989) and *P.monodon* (Yashiro, 1989). 17-a HP significantly increased diameter of oocyte in *P.vannamei* ovaries *in vitro*, but not by progesterone and 17-a-b di HP (Tsukimura and Kamemoto,1988,1991). Koskela *et al.* (1992) noticed no significant increase in ovarian development or GSI following administration of 17-a HP to female *P.esculentus*. Progesterone failed to effectively evoke any ovarian growth in preadult *P.monodon* whereas ESA did (Anon.,1992). Progesterone and estradiol stimulated yolk synthesis in *P.vannamei* ovaries, *in vitro* (Quackenbush,1992).

Sarojini *et al.*(1986) registered increased oogenesis in estrone and estrogen injected *M.lamerrii*. Administration of exogenous steroids (estradiol and estrone) caused increased ovarian development over control crabs, *S.serrata* (Sarojini *et al.*,1990). Tsukimura and Kamemoto (1991) found no increased diameter of oocyte in *P.vannamei* ovary *in vitro* by applying 17-b estradiol. Similar result was also reported by Koskela *et al.*(1992) in *P.esculentus* and Anon.(1992) in *P.monodon*.

Bomirski and Klek-Kawinska (1976) reported that HCG administration stimulated oogenesis in *Crangon crangon*. Similar observations had been made in the crab, *Thalamita crenata* by Oyama and Kamemoto (cited by Kulkarni *et al.*,1979) and in the isopod, *Idotea*

balthica (Souty and Picaud,1984) while studying the impact of HCG on oogenesis.

Sarojini and Persis (1988) recorded stimulation of vitellogenesis in *Caridina rajadhari* by

the treatment of the same steroid hormone.Yano and Wyban (1987) received more

advanced ovarian development and spawning from *P.vannamei* females treated with HCG.

Yano and Chinzei (1991) reported that a significant increase in Vg concentration in the

serum of ESA *P.japonicus* females injected with HCG. Zukowska-Arendarczyk (1981)

proposed that both follicle stimulating hormone (FSH) and leutinizing hormone (LH) have

stimulatory effect on the ovary development of *Crangon crangon*. Nagabhushanam *et*

al.(1987) used FSH and LH for induced spawning in *Caridina rajadhari*.

Sambasivarao *et al.* (1985) recorded stimulation of oogenesis in *Metapenaeus affinis*

by steroid hormones and Sasikala and Subramoniam (1987) tested the efficacy of various

mammalian steroid hormones with encouraging results in *Paratelphusa hydrodromous*.

Mendoza-Alfaro (1992) provoked vitellogenesis by steroid hormones in *in vitro* incubated

P.vannamei ovaries.

The AG of testosterone injected *Parapenaeopsis hardwickii* and *P. stylifera*

(Nagabhushanam and Kulkarni,1981; Nagabhushanam *et al.*,1987) showed hypertrophy

and hyperplasia. Sarojini *et al.*(1993) provided evidence that 5-HT stimulated testicular

maturation of *Uca pugilator* and they hypothesized that 5-HT functions indirectly to

stimulate release of GSH; in turn GSH acts directly on the AG to release AGH which

subsequently acts on the testis to bring about testicular maturation (Sarojini*et al.*,1994).

Tsukimura and Kamemoto (1991) reported increased oocyte size in *P. vannamei* treated with JH III and MF. Mendoza-Alfaro (1992) was able to obtain vitellogenesis in *P.vannamei* ovaries *in vitro* with MO extracts. Richardson *et al*.(1991) demonstrated that 5-Hydroxy Tryptamine (5-HT), a neurotransmittor stimulates release of GSH and thereby increasing ovarian development in *U.pugilator* and the same was also reported by Kulkarni and Fingerman (1992) and Kulkarni *et al*.(1992) in *U.pugilator* and *P.clarkii* respectively.

III MATERIAL AND METHODS

The experiment was conducted for 45 days during April- June, 1994, following the method of Yano (1985) on induced ovarian maturation of *Metapenaeus ensis* by injecting progesterone through the first abdominal segment of the shrimp.

3.1 Maturation room

The experiment was conducted in a separate shed the inside of which was covered all over with black plastic sheet in order to give darkness and to minimize disturbance. There was provision for water supply, aeration and lighting in the shed (Plate I).

3.2 Experimental tanks

Round cement cisterns of 45 cm diameter and 60 cm height were used for the experiment. The inside of the tank was painted black. Aeration points were provided for continuous aeration in the tanks. Tiles were provided uniformly in all the tanks to offer shelter for the weak shrimps during moulting and to avoid cannibalism.

3.3 Experimental animals

Farm reared *Penaeus indicus* female specimens were brought to the experimental shed from the Matsyafed farm at Narakkal. The age of the animals was 4.5 months and only the individuals having a minimum size of 130 mm were used for the experiment.

Plate I A view of maturation room

The animals were acclimatized to the tank conditions for 7 days before being subjected to injections and were given commercial pellet feed during this period.

3.4 Feed

The commercial pellet feed obtained from Higashimaru Feeds (India) Ltd., Kochi was used for feeding purpose during the experiment.

The biochemical composition of the feed is as follows,

crude protein   - 36 %

crude fat   - 6 %

ash   - 15 %

moisture   - 10.5 %

3.5 Experimental material

Progesterone (Paines and Byrne,U.K.) and Tocopherol (Merck, Bombay)were used for the injection. They were diluted with ethyl alcohol to get different concentrations for the test. A micro-litre syringe (Top,Bombay) was used for the injection of the test material into the shrimps (Plate II).

3.6 Experimental procedure

3.6.1 Preparation of test material.

Progesterone was diluted with ethyl alcohol to get three different test concentrations (0.05 ug/ul, 0.1 ug/ul and 0.2 ug/ul). Tocopherol (vitamin E) was also diluted with ethyl alcohol to get 0.05 ug/ul, 0.1 ug/ul and 0.2 ug\ul concentrations.

Plate II Micro-litre syringe
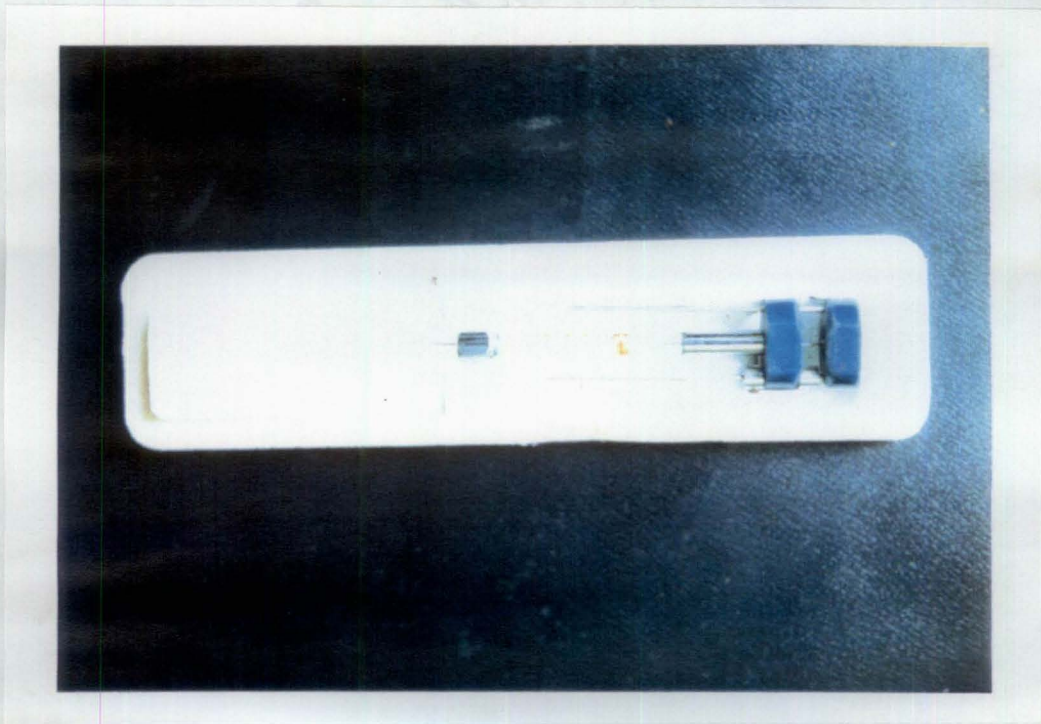
For making the mixture of progesterone and tocopherol the solutions of progesterone and tocopherol were mixed in 7:3 ratio (V/V).

3.6.2 Administration of test material.

The test material, viz., three concentrations of progesterone, progesterone-tocopherol mixture and ethyl alcohol (control) were injected into the shrimps using a micro-litre syringe at the first abdominal segment following the method of Yano (1985).

The weighed shrimps were given a single dose each of the three concentrations of the test material. The doses tried were 0.05ug/g, 0.1ug/g and 0.2ug/g body weight (bw) of the animal and control shrimps with the above mentioned doses of ethyl alcohol on a volumetric (ul) basis. The dose of each material administered is given in the Figures 1-3.

3.6.3 Maturation tank management.

3.6.3.1 Stocking.

Each tank was stocked with 10 numbers of the shrimps which were given identical treatment both in test material as well as in its dosage. The water level in the tanks was kept above 50 cm and the tanks were covered with plastic-mesh nets to prevent escape of the shrimps by jumping. Tiles were provided at the bottom of the tanks to provide shelter for the weak and moulting shrimps and to minimize cannibalism among the test animals.
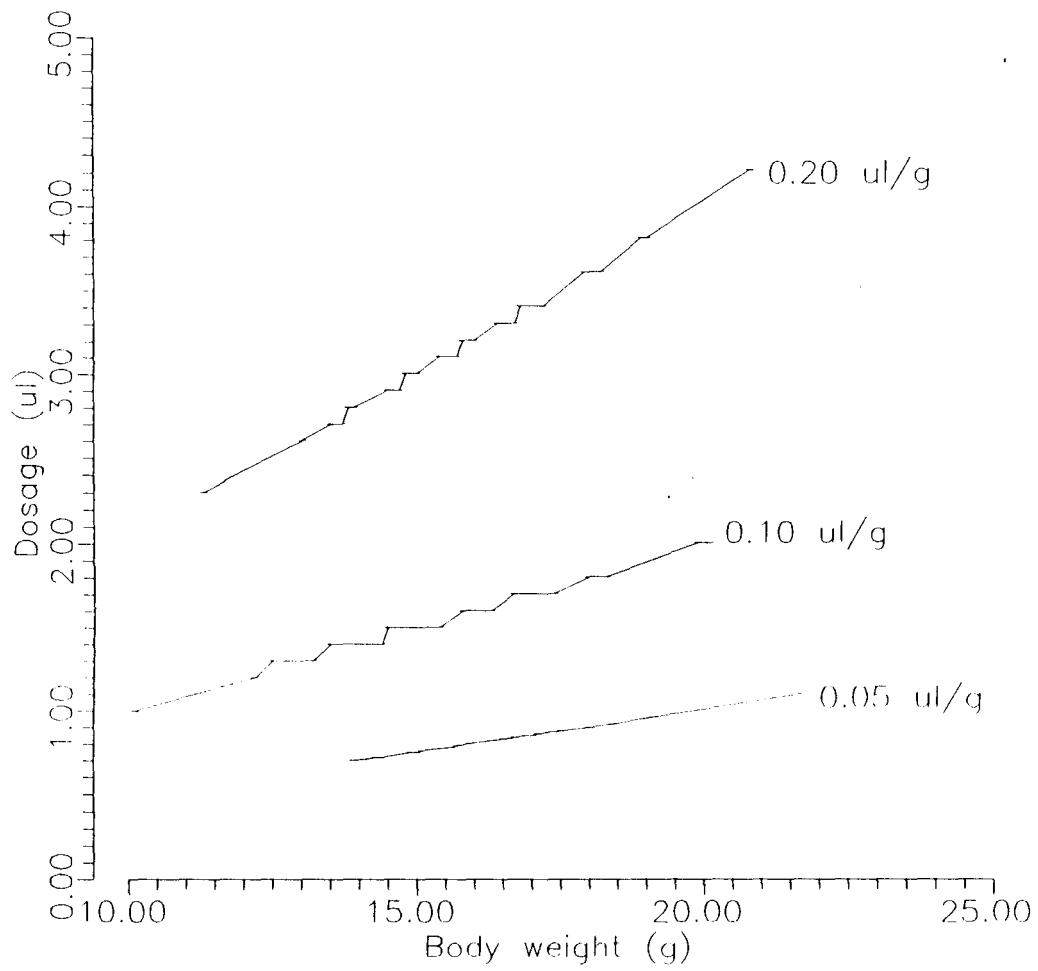
Fig. 1 Dosage of alcohol for control shrimps
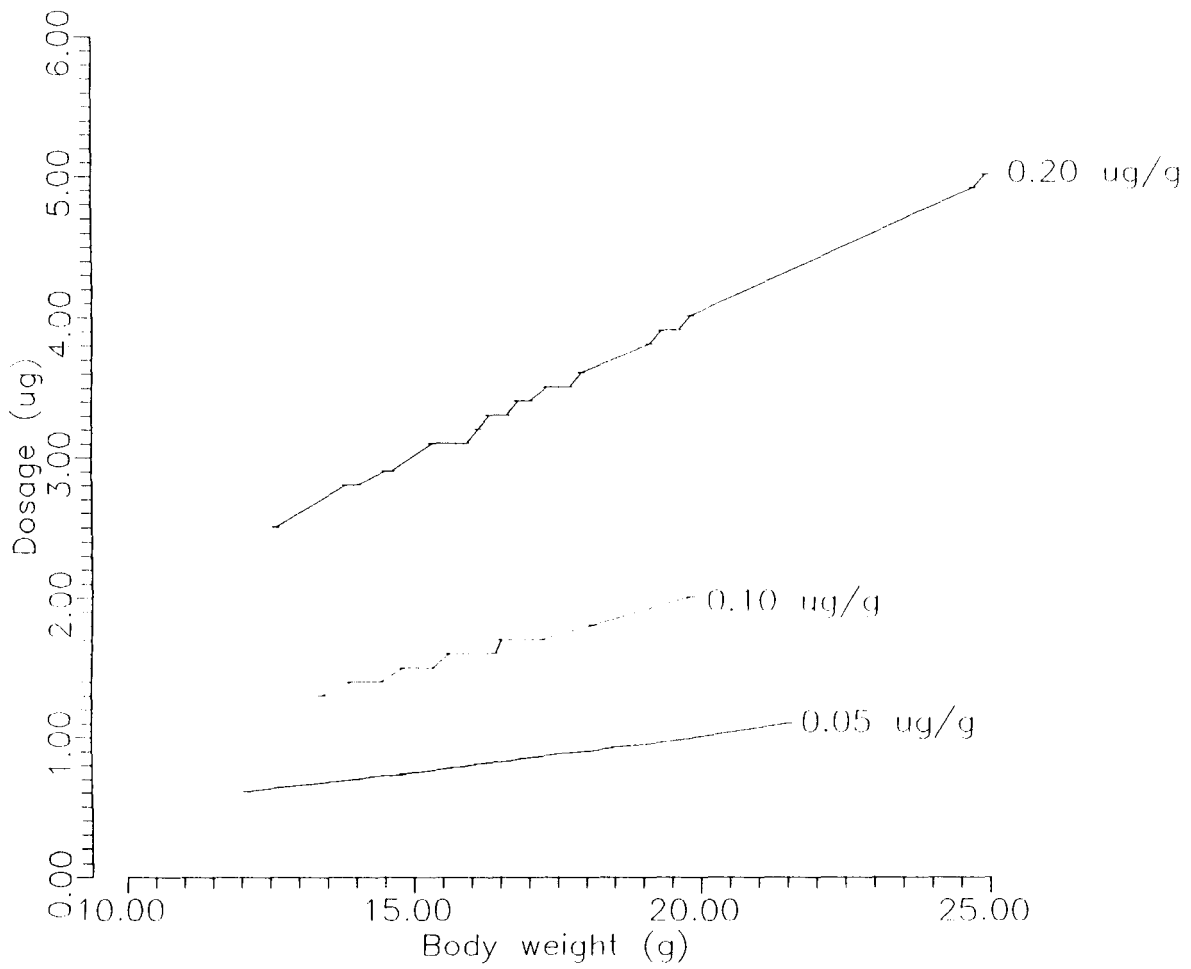
Fig. 2 Dosage of progesterone
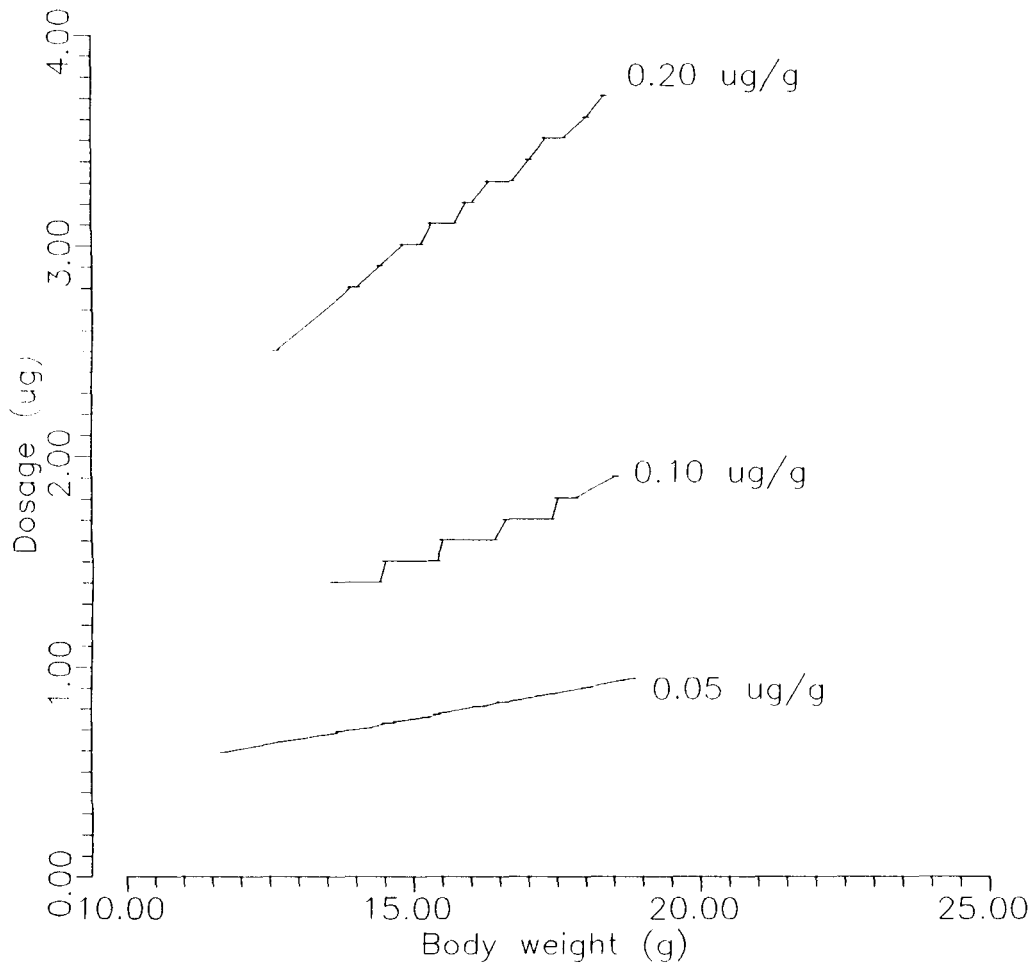
Fig. 3   Dosage of progesterone plus tocopherol

3.6.3.2 Feeding.

Feed was given *ad libitum* in a small tray kept near the shelter tile. Feeding was done twice a day, one during the morning, after removing the left over feed, and the other during the evening.

3.6.3.3 Water quality maintenance.

Continuous mild aeration was provided to ensure sufficient amount of dissolved oxygen and also to drive out noxious gases, if present. About 70 % of the water in the tank was exchanged once in three days by siphoning and refilling. The exuviae, feed remnants, etc. were removed during the water exchange. The animals were closely inspected for the development of the ovary, general health, as well as to asses their number. The dead ones were removed as and when noticed. The photoperiod was regulated at 16D:8L, using artificial light.

3.6.4 Determination of water quality parameters.

The water quality parameters like temperature, salinity and pH were measured periodically.

The ranges were,

Temperature   - 26.1 - 29.2° C.

Salinity   - 24.3 - 25.7 %. .

pH   - 8.0 - 8.1.

3.7 Evaluation of results

3.7.1 Visual assessment of ovarian development.

Visual observation through the dorsal integument of the shrimp was done to assess the ovarian development periodically, during the experimental period using a torch-light focusing light from the ventral side of the animal.

3.7.2 Gonado-somatic index (GSI).

The animals were dissected after taking the body weight and was ovary taken out and weighed. The Initial GSI was obtained from 27 shrimps sacrificed at the start of the experiment and all the shrimps remaining in the tankswere dissected to get the final GSI.

$$\text{Gonado-somatic index} = \frac{\text{Weight of the gonad}}{\text{Weight of the animal}} \times 100$$

3.7.3 Somatic growth.

The percentage somatic growth (PSG) is used to compare the somatic growth under different treatments which is calculated by using the following formula,

$$\text{PSG} = \frac{\text{Final body weight - Initial body weight}}{\text{Initial body weight}} \times 100$$

3.7.4 Ovarian growth (OG).

The average weight of the ovaries collected from 27 shrimps at the beginning of the experiment and average weight of the ovaries taken from shrimps remaining in each tank, were used for the calculation of ovarian growth using the formula,

Ovarian growth = Final weight of ovary- Initial weight of ovary

3.7.5 Survival rate.

The percentage survival rate is used for the comparison of survival rate.

Percentage survival rate = $\dfrac{\text{Number of animals harvested}}{\text{Number of animals introduced.}}$ X 100

3.8 Statistical design of the experiment

3.8.1 Layout of the experiment.

Completely randomized design (CRD) was used for conducting the experiment. The three test solutions were used each at three different dosages; thus there were nine treatments. Each treatment was replicated thrice. So the experiment was conducted in 27 tanks. Each tank was marked as either C (control) or P (progesterone) or T (mixture of progesterone and tocopherol) followed by 0.05, 0.1 and 0.2 to represent the dosages and replication number as subscripts, after distributing the treatments randomly.

3.8.2 Analysis of the results.

The Analysis of variance (ANOVA) technique was used for analyzing results after making angular transformations wherever found necessary (Snedecor and Cochran, 1968).

The Gonado somatic index data were also compared using the ANOVA technique after making necessary transformation of the data.

For the comparison of Percentage somatic growth, the transformed data were subjected to ANOVA.

The Students' `t' test was used for finding the difference between the initial ovarian weight and final ovarian weight, and ANOVA for comparing the difference in ovarian weight (Ovarian growth).

The correlation technique was used for finding the relationship between the somatic growth and ovary growth.

The data for survival were transformed before carrying out the ANOVA.

IV RESULTS

The results of the experiment can be considered conveniently under the following heads.

1).Survival rate,

2). Visual observation of ovarian development,

3).Analysis of gonado somatic index,

4).Ovarian growth,

5). Somatic growth

6).Correlation between somatic growth and ovarian growth.

4.1 Survival rate

Generally the survival rate was high and it was 80 % or more except in one of the tanks of $P_{0.10}$ whose survival rate was 70 %. The average in each of the treatments was above 83 % and overall survival rate was 89.63 % (Table 2). As can seen from Table 3 there was no significant difference in survival rate among the treatments. The average percentage survival rate obtained is given in Fig.4.

4.2 Visual assessment of ovarian development

The shrimps were subjected to periodical observation of ovarian growth through the dorsal integument during the course of the experiment. But there was no marked visible change in ovary development during the experiment. So for a closer inspection

Table 2 Percentage survival data for ANOVA treatment

Treatments	Replications			Total	Average
	I	II	III		
$C_{0.05}$	80	100	100	280	93.3
$C_{0.10}$	80	80	90	250	83.3
$C_{0.20}$	90	80	100	270	90.0
$P_{0.05}$	90	80	100	270	90.0
$P_{0.10}$	90	90	70	250	83.3
$P_{0.20}$	80	90	80	250	83.3
$T_{0.05}$	90	100	100	290	96.6
$T_{0.10}$	100	90	80	270	90.0
$T_{0.20}$	100	90	100	290	96.6
Total				2420	89.63

Table 3. ANOVA for percentage survival rate

Source of variation	Degrees of freedom	Sum of squares	Mean S.S.	F
Treatment	8	1262.2659	157.7832	1.2517
Error	18	2268.9343	126.0519	
Total	26	3531.2002		
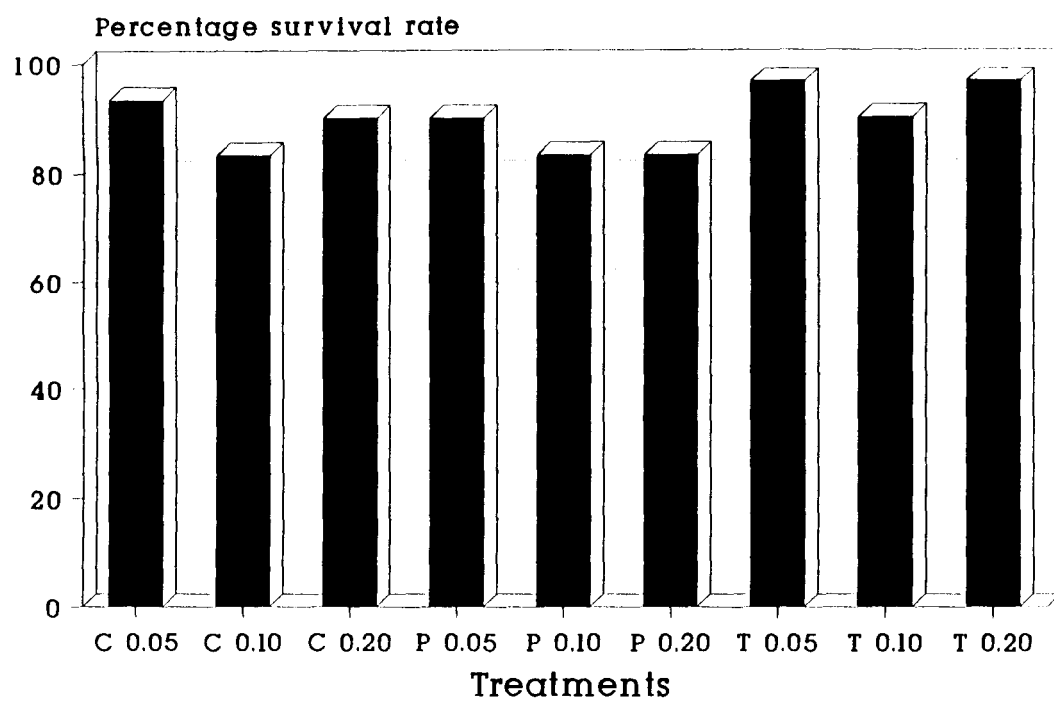
Fig.4 Percentage survival rate for different treatments

of ovary, shrimps were dissected at the end of the experiment. The ovary was somewhat

thick, yellowish -white with bluish-green patches all over during the final stage, in

contrast to the initial thin transluscent ovary. The difference in size and colour was more

prominent in the case of progesterone (P) and progesterone combined with tocopherol

(P+T) treated shrimps (Plate III), in comparison to the control shrimps.

4.3Analysis of gonado somatic index (GSI)

The highest GSI value was obtained for the shrimps treated with progesterone at

0.20 ug /body weight of the shrimp, viz., $P_{0.20}$ (0.771), followed by $P_{0.10}$ (0.677) and $T_{0.20}$

(0.630) and the lowest values for the control shrimps (0.384, 0.407, 0.514) (Table 4 and

Figs.5-13). The initial GSI of 27 shrimps is given in the Fig. 15 which was low compared

to the shrimps after the experiment irrespective of treatments.

The comparison of GSI values using ANOVA technique showed significant

difference among the treatments at 5% level (Table5).

On pairwise comparison it was found that GSI values for $P_{0.20}$ and $P_{0.10}$ stand apart

from other values, and also these values are well above the GSI values of the control

shrimps.

The GSI values of $P_{0.20}$ and $P_{0.10}$ fall in the same group and there is close

relationship between the GSI values of progesterone and progesterone combined with

tocopherol treated shrimps except in the case of $T_{0.05}$ (0.576) and it is also inferred that

these treatments ($P_{0.20}$ and $P_{0.10}$) differ significantly from controls (Fig. 14).

Table 4. Average GSI values for ANOVA treatment

Treatments	Replications			Total	Average
	I	II	III		
$C_{0.05}$	0.410	0.315	0.496	1.221	0.407
$C_{0.10}$	0.380	0.349	0.424	1.153	0.384
$C_{0.20}$	0.513	0.476	0.553	1.542	0.514
$P_{0.05}$	0.647	0.693	0.529	1.869	0.623
$P_{0.10}$	0.686	0.696	0.649	2.031	0.677
$P_{0.20}$	0.764	0.760	0.789	2.313	0.771
$T_{0.05}$	0.557	0.473	0.697	1.727	0.576
$T_{0.10}$	0.604	0.543	0.625	1.772	0.591
$T_{0.20}$	0.531	0.722	0.638	1.891	0.630
Total				15.518	

Table 5 ANOVA table for GSI

Source of variation	Degrees of freedom	Sum of squares	Mean S.S.	F
Treatment	8	0.3682	0.0460	9.73*
Error	18	0.0857	0.0082	
Total	26	0.4539		

* Significant difference at 5 % level.

The critical difference value at 5 % level for the pairwise comparison was calculated to be 0.0971.

Pairwise comparison of GSI values

$C_{0.10}$ 0.384

$C_{0.05}$ 0.407

$C_{0.20}$ 0.514

$T_{0.05}$ 0.576

$T_{0.10}$ 0.591

$P_{0.05}$ 0.623

$T_{0.20}$ 0.630

$P_{0.10}$ 0.677

$P_{0.20}$ 0.771
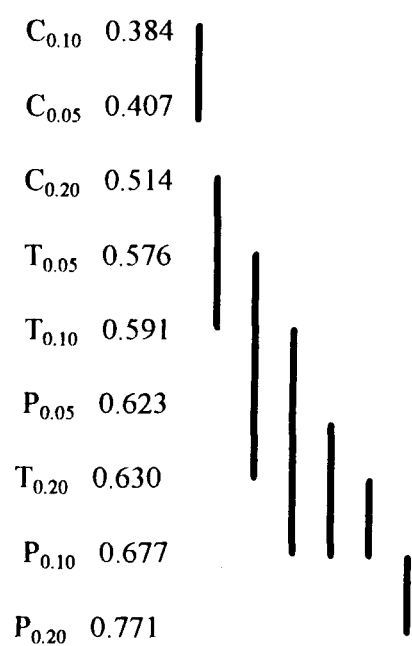
The treatments which are not significantly different are connected with vertical lines.

Plate III The ovary of progesterone treated shrimp after the experiment
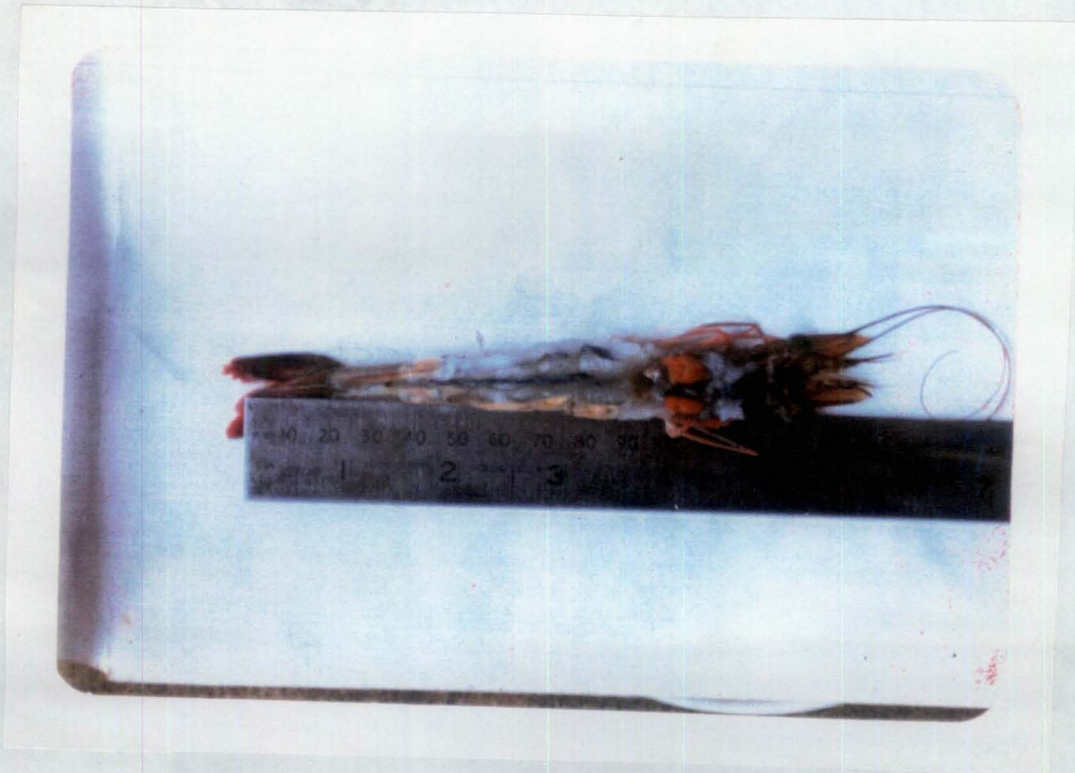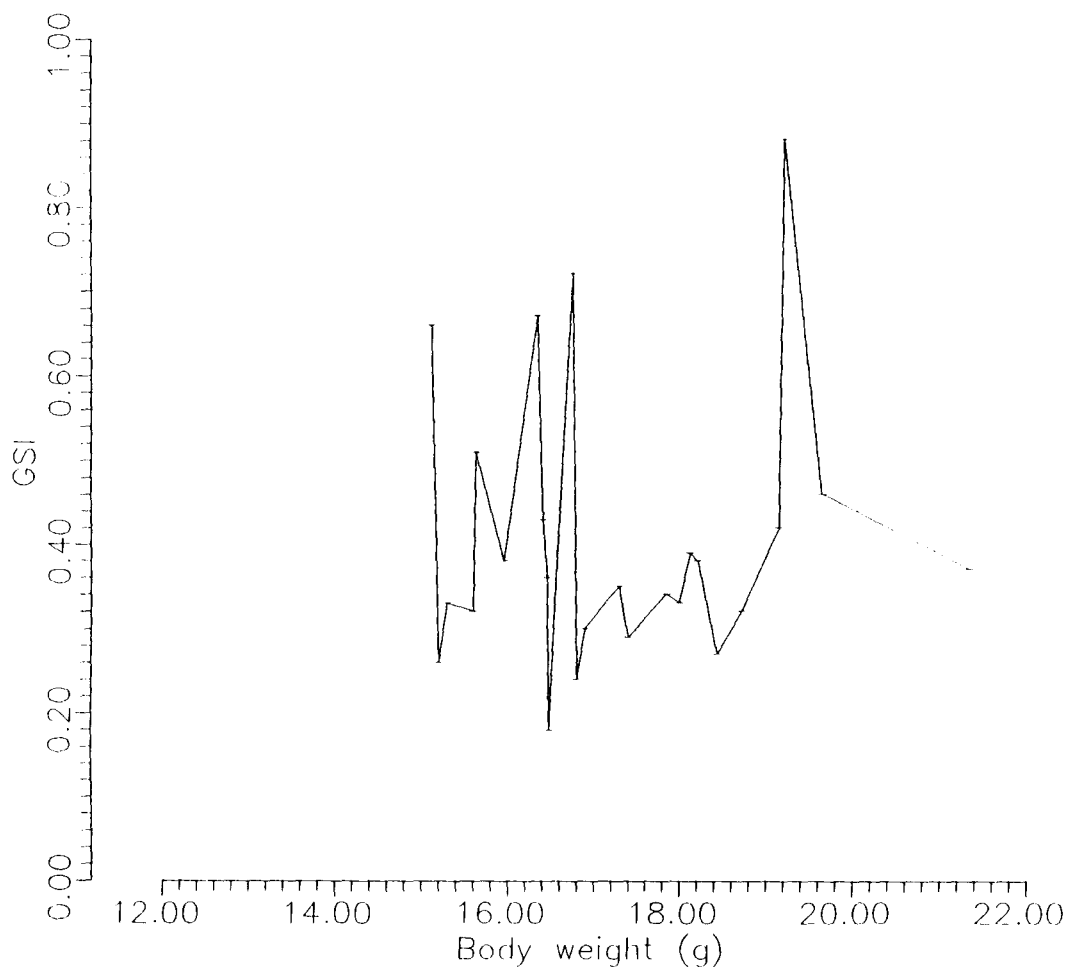
Fig.5 Gonado somatic index for $C_{0.05}$
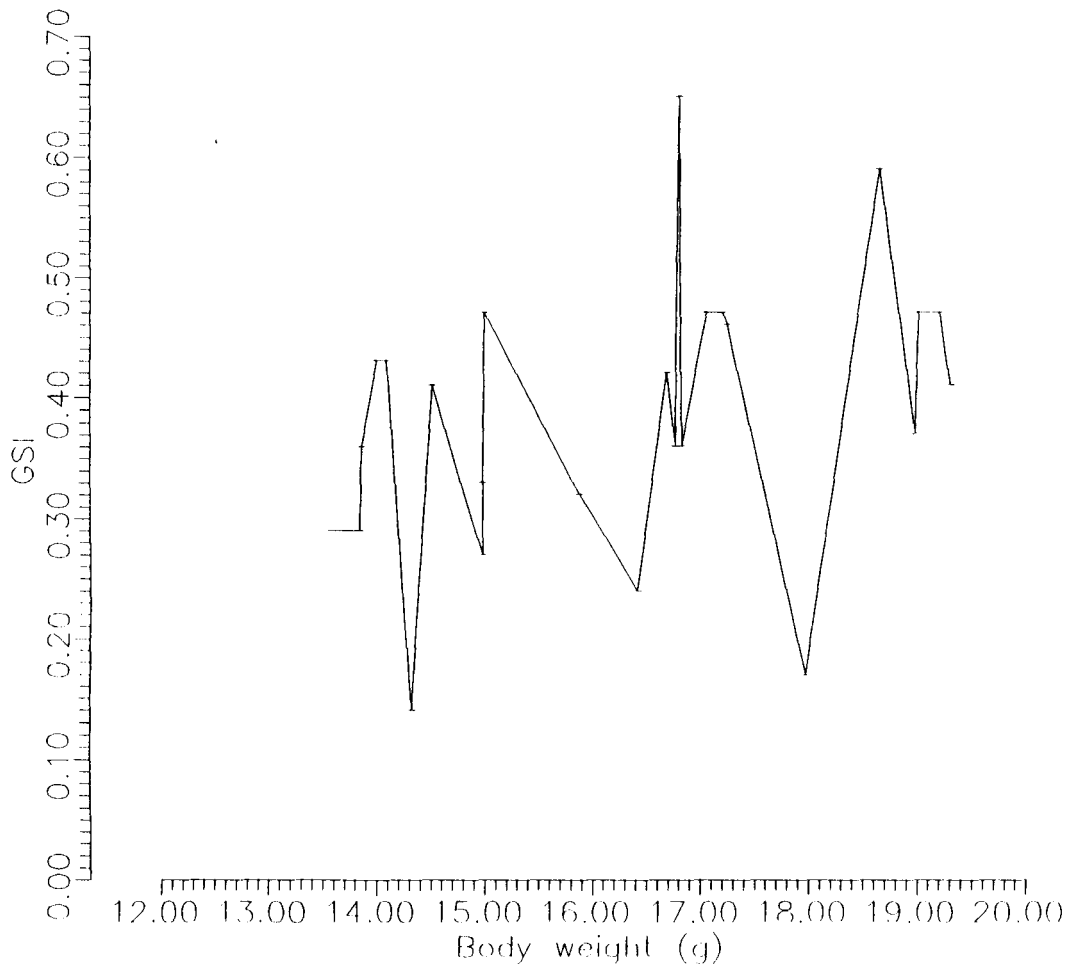
Fig.6 Gonado somatic index for $C_{0.10}$
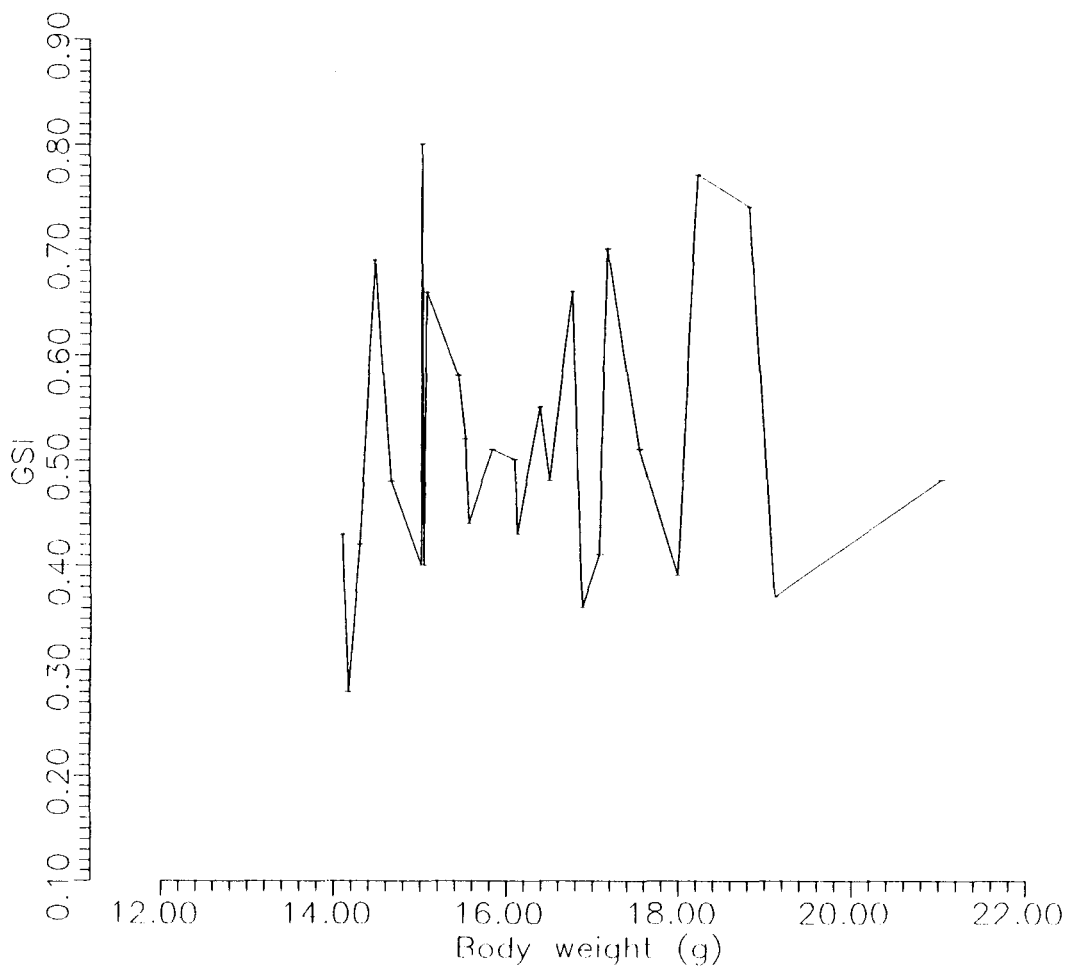
Fig.7 Gonado somatic index for $C_{0.20}$
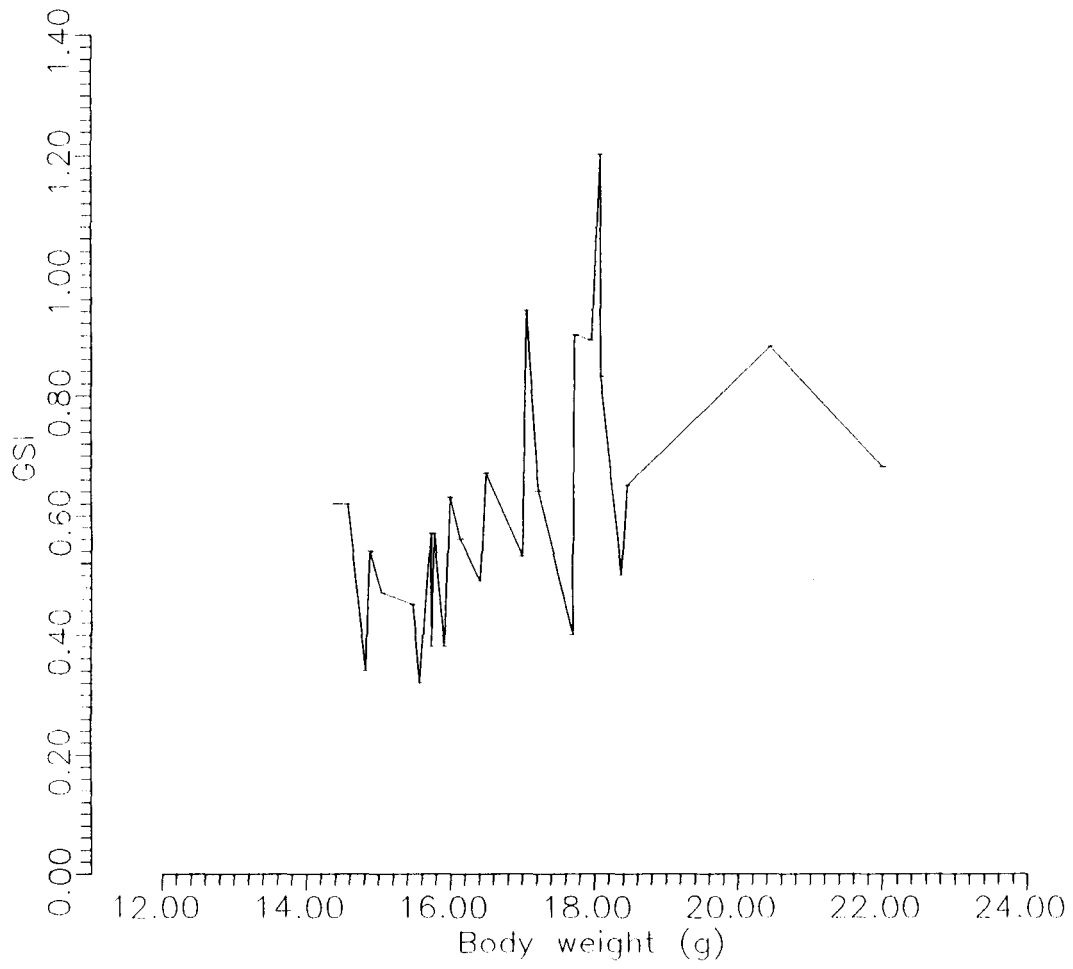
Fig.8 Gonado somatic index for $P_{0.05}$
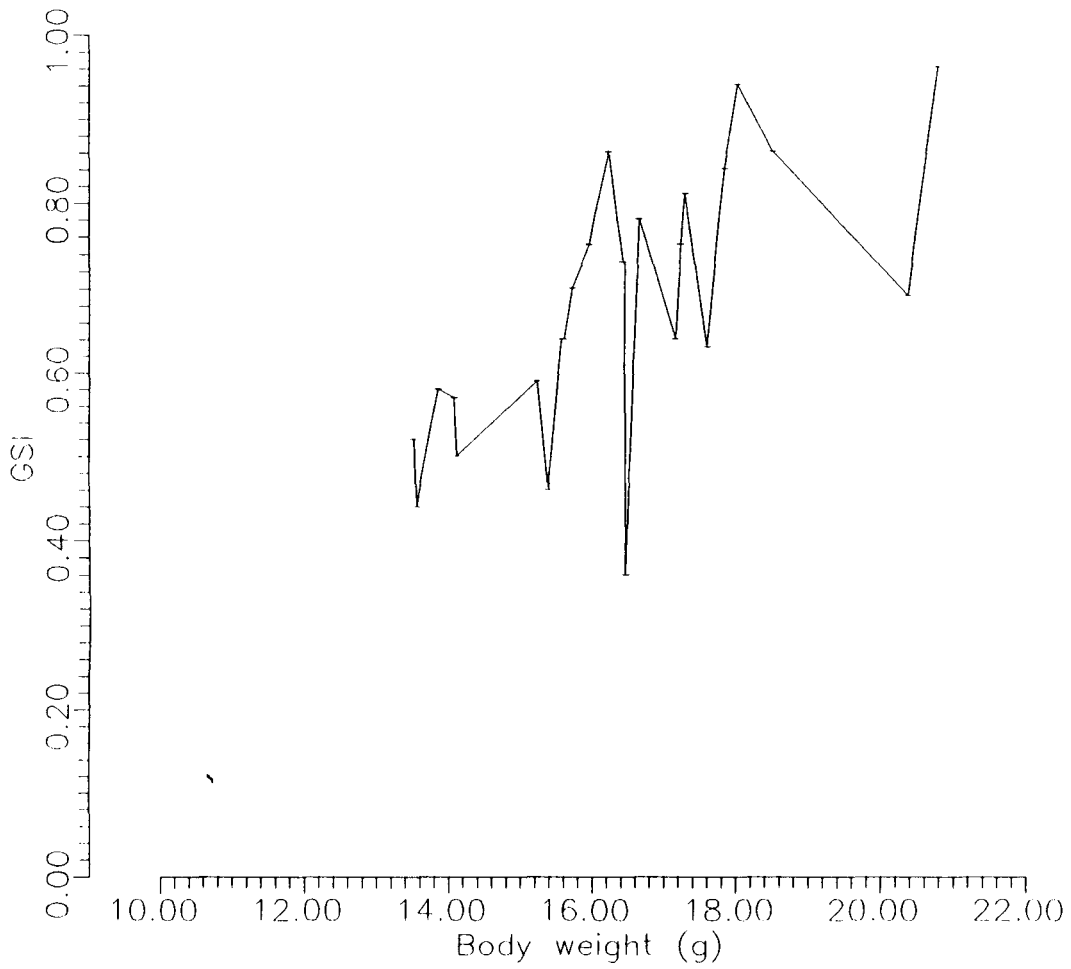
Fig.9 Gonado somatic index for $P_{0.10}$
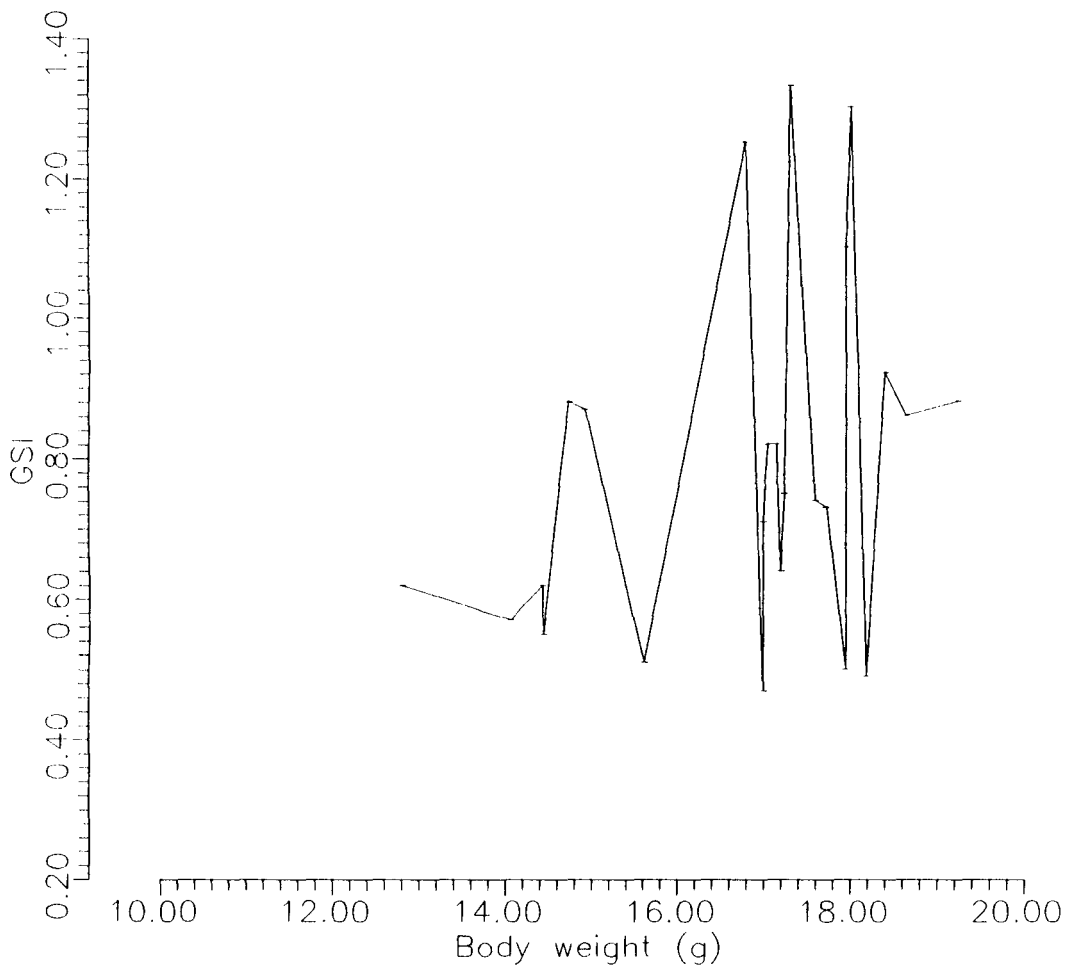
Fig.10 Gonado somatic index for $P_{0.20}$
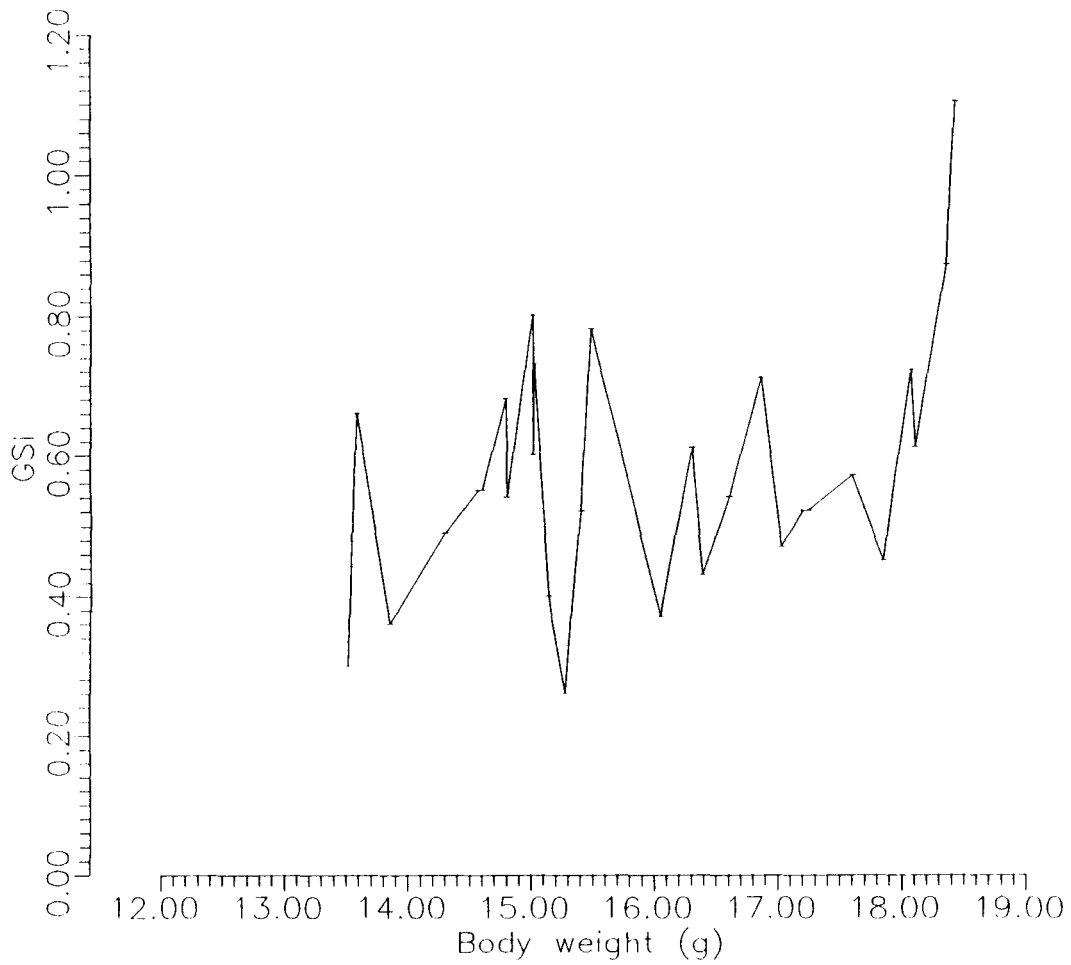
Fig.11 Gonado somatic index for $T_{0.05}$
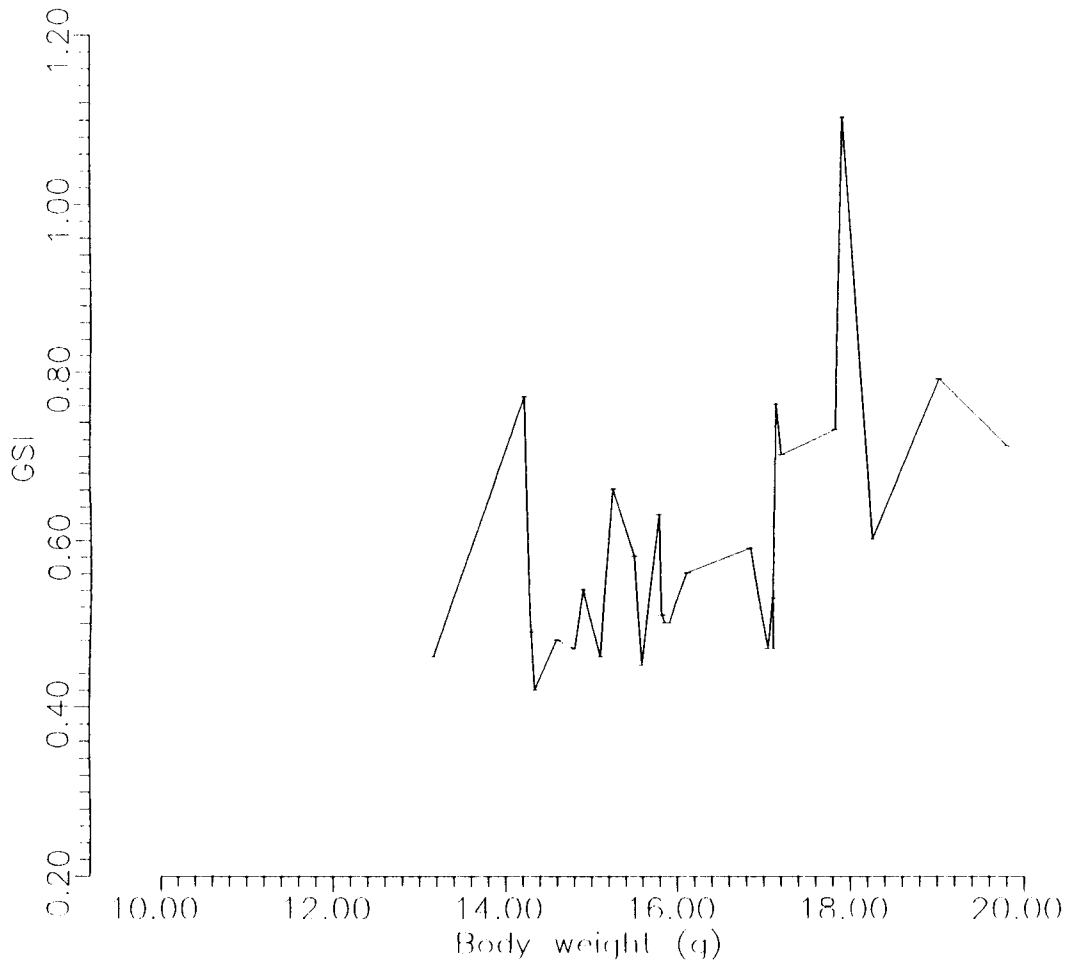
Fig.12 Gonado somatic index for $T_{0.10}$
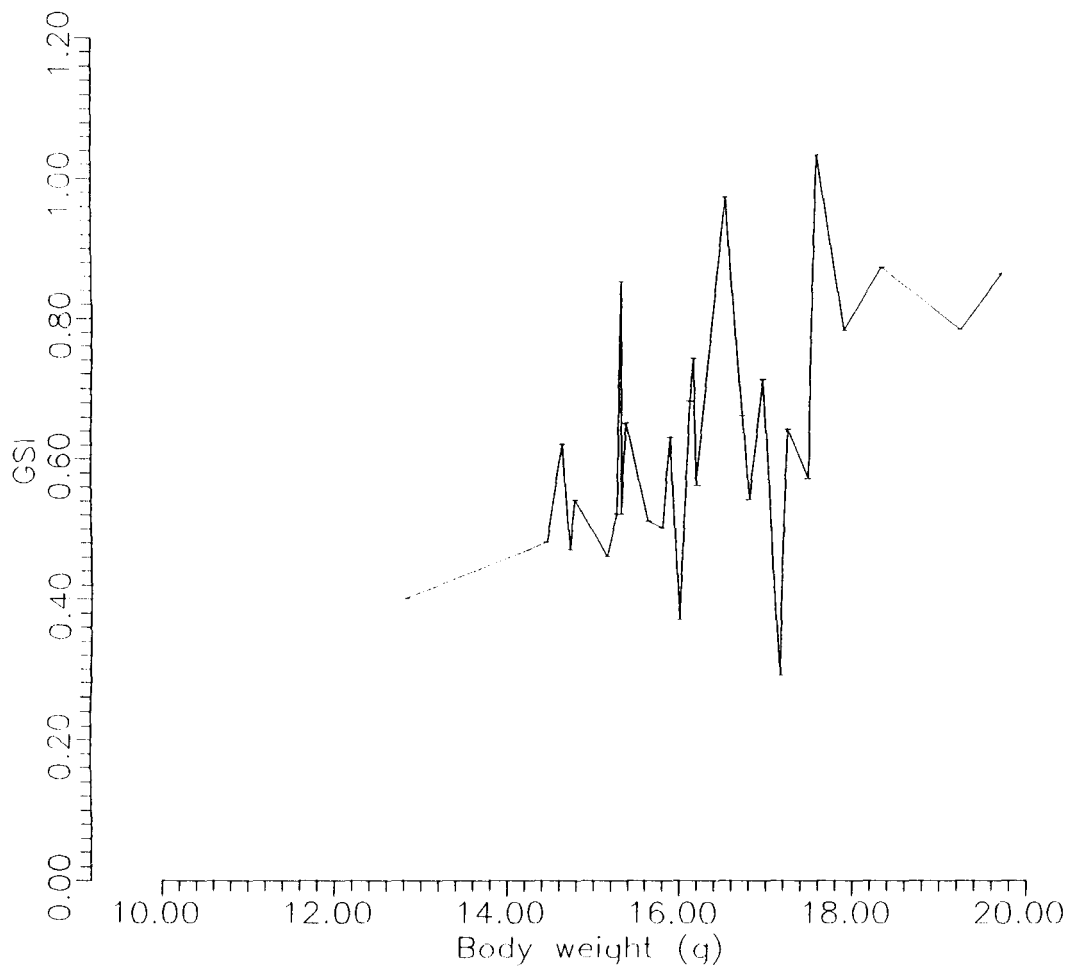
Fig.13 Gonado somatic index for $T_{0.20}$
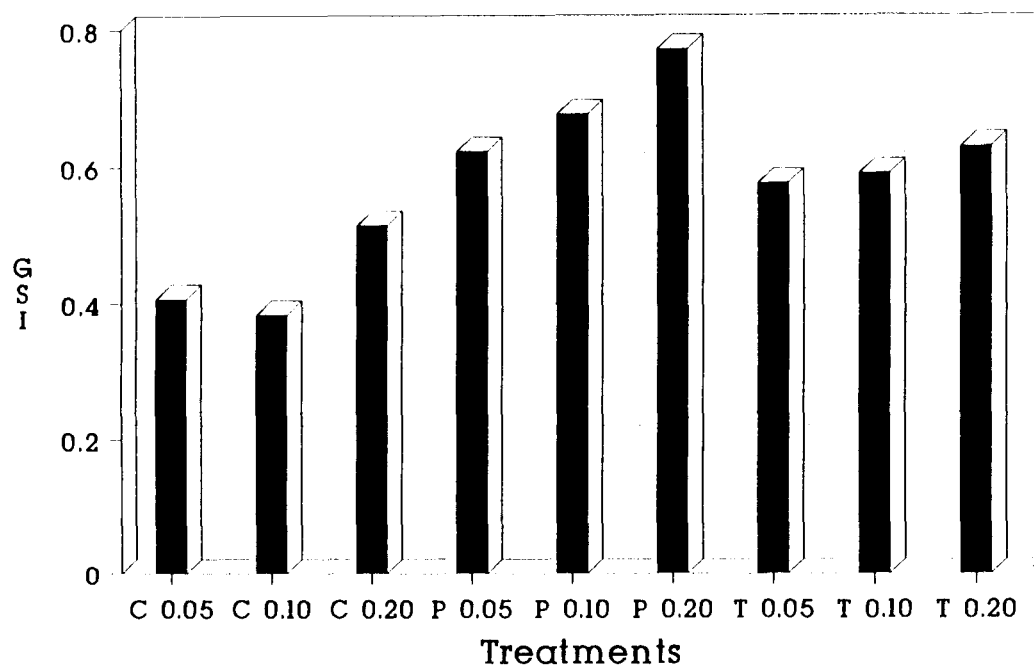
Fig. 14 GSI values for different treatments
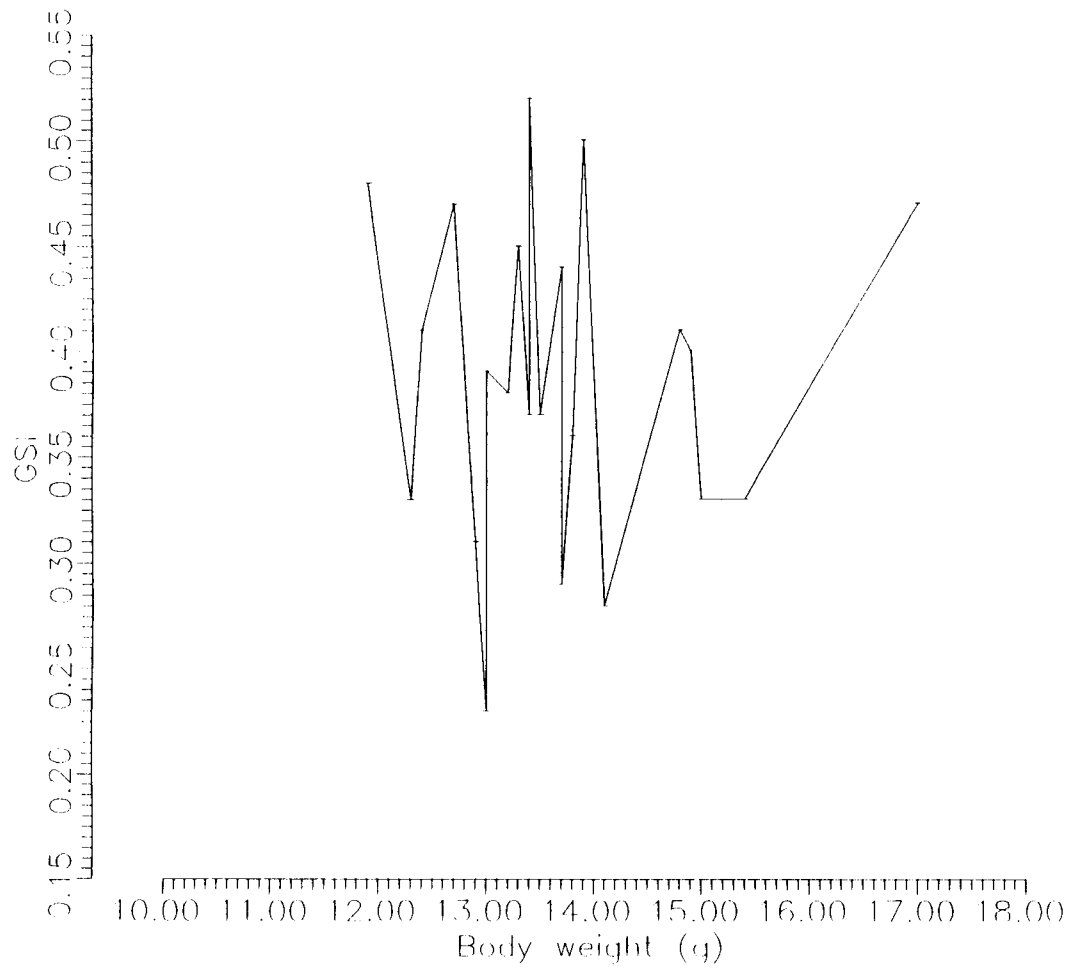
Fig.15 Initial gonado somatic index

The controls $C_{0.05}$ and $C_{0.10}$ form a unique group of low GSI values which clearly indicates the difference from that of P and P+T treated shrimps. The $C_{0.20}$ and $T_{0.05}$ make a group which in turn group with $T_{0.10}$ and $P_{0.05}$ which have also low GSI values compared to the high GSI values of $P_{0.20}$, $P_{0.10}$ and $T_{0.20}$.

4.4 Somatic growth (SG)

The percentage somatic growth (PSG) under various treatments were also compared. Generally the control group showed high average PSG ($C_{0.05}$ = 6.5767, $C_{0.10}$ =6.3067, and $C_{0.20}$ =4.2833) compared to the P and P+T treated shrimps except in the case of $T_{0.05}$ (4.0167) and the lowest value is obtained for the progesterone treated shrimps especially $P_{0.20}$ (0.5200) and $P_{0.10}$ (1.78). The $T_{0.20}$ has the third lowest PSG value (2.0033) followed by $T_{0.10}$ (2.9000) (Tables 6 and 7). The ANOVA comparison showed significant difference at 5% level (Table 8).

The critical difference analysis revealed that the treatments $P_{0.20}$ and $P_{0.10}$ have got exceptionally low PSG compared to the rest of the treatments. The treatment $P_{0.10}$ formed a class of low PSG with $T_{0.20}$. The treatments $P_{0.05}$ $T_{0.05}$ and $T_{0.20}$ formed a group of slightly high PSG. The control values grouped together for a high PSG (Fig. 16).

4.5 Ovarian growth (OG)

The students 't' test was used for the comparison of initial ovarian weight and final ovarian weight (Fig. 17). It showed that there is significant differences between the initial ovarian weight and final ovarian weight except in the case of treatments $C_{0.05}$ and

Table 6. Percentage somatic growth

Treatments	Replications	Average body weight (g)		Growth (g)	Growth (%)
		Initial	Final		
$C_{0.05}$	I	17.06	18.19	1.13	6.62
	II	15.55	16.80	1.25	8.04
	III	16.56	17.40	0.84	5.07
$C_{0.10}$	I	16.47	17.71	1.24	7.53
	II	13.74	15.00	1.26	9.17
	III	15.80	16.15	0.35	2.22
$C_{0.20}$	I	16.76	17.28	0.52	3.10
	II	14.72	15.62	0.90	6.11
	III	15.38	15.94	0.56	3.64
$P_{0.05}$	I	16.18	16.34	0.16	0.99
	II	17.21	17.83	0.62	3.36
	III	16.03	16.34	0.31	1.93
$P_{0.10}$	I	15.63	16.12	0.49	3.13
	II	16.59	16.79	0.20	1.21
	III	16.05	16.21	0.16	1.00
$P_{0.20}$	I	16.59	16.70	0.11	0.66
	II	16.36	16.45	0.09	0.55
	III	17.14	17.20	0.06	0.35
$T_{0.05}$	I	15.69	16.12	0.43	2.74
	II	15.46	15.85	0.39	2.52
	III	14.87	15.88	1.01	6.79
$T_{0.10}$	I	15.49	15.86	0.37	2.39
	II	15.89	16.60	0.71	4.47
	III	15.75	16.04	0.29	1.84
$T_{0.20}$	I	16.12	16.60	0.48	2.98
	II	16.17	16.40	0.23	1.42
	III	15.53	15.78	0.25	1.61

Table 7. Percentage somatic growth data for ANOVA treatment

Treatments	Replications			Total	Average
	I	II	III		
$C_{0.05}$	6.62	8.04	5.07	17.73	6.5767
$C_{0.10}$	7.53	9.17	2.22	18.92	6.3067
$C_{0.20}$	3.10	6.11	3.64	12.85	4.2833
$P_{0.05}$	0.99	3.36	1.93	6.28	2.0933
$P_{0.10}$	3.13	1.21	1.00	5.34	1.7800
$P_{0.20}$	0.66	0.55	0.35	1.56	0.5200
$T_{0.05}$	2.74	2.52	6.79	12.05	4.0167
$T_{0.10}$	2.39	4.47	1.84	8.70	2.9000
$T_{0.20}$	2.98	1.42	1.61	6.01	2.0033

Table 8. ANOVA table for percentage somatic growth

Source of variation	Degrees of freedom	Sum of squares	Mean S.S.	F
Treatment	8	103.5539	12.9442	3.9842*
Error	18	58.4795	3.2489	
Total	26	162.0334		

* Significant difference at 5 % level.

The critical difference for comparing the treatments was 2.5519 at 5 % level.

Pairwise comparison of treatments

$P_{0.20}$ 0.5200

$P_{0.10}$ 1.7800

$T_{0.20}$ 2.0033

$P_{0.05}$ 2.0933

$T_{0.10}$ 2.9000

$T_{0.05}$ 4.0167

$C_{0.20}$ 4.2833

$C_{0.10}$ 6.3067

$C_{0.05}$ 6.5767
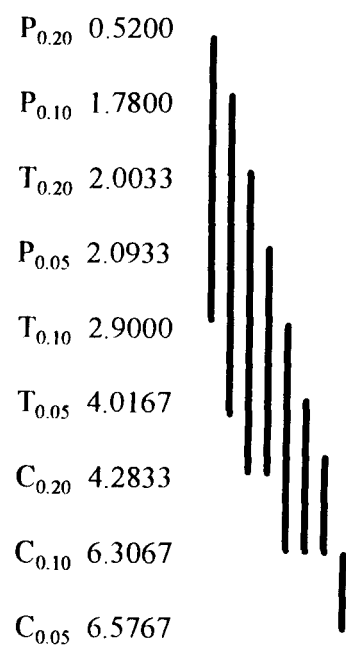
The vertical lines denote no significant difference between the treatments.
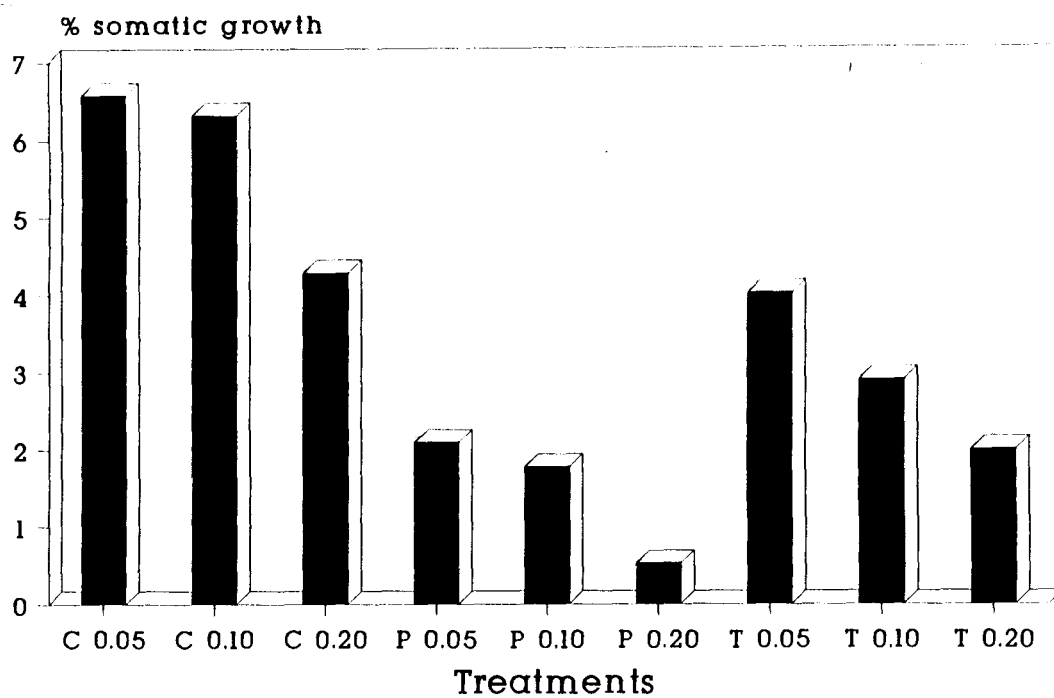
Fig. 16 Percentage somatic growth for different treatments

$T_{0.10}$ (Table 11) and ovary growth was significantly different at 5% level (Tables 9 and 10).

The comparison of the ovarian weights between the treatments by ANOVA technique and critical difference gave the following results,

1) The gonadal growth of $P_{0.20}$ (0.0781) was the highest among the treatments and stood separately from the rest.

2) The gonadal growth decreased with decrease in the quantity of progesterone administered viz., $P_{0.20}$, $P_{0.10}$, $P_{0.05}$, $T_{0.20}$, $T_{0.10}$ and $T_{0.05}$, and

3) Lowest values were obtained for the control shrimps ($C_{0.20}$ = 0.0281, $C_{0.10}$ = 0.0184 and $C_{0.05}$ = 0.0111) (Fig. 18).

4.6 Correlation between somatic growth and ovarian growth

The Table 12 gives the values of absolute growth rate and gonadal growth. It was noticed that there exists a negative correlation between the somatic growth and gonadal growth (- 0.97) (Fig.19).

Table 9. Data of ovary growth for ANOVA treatment

Treatments	Replications			Total	Average
	I	II	III		
$C_{0.05}$	0.0218	0.0008	0.0338	0.0564	0.0188
$C_{0.10}$	0.0168	0.0008	0.0168	0.0344	0.0115
$C_{0.20}$	0.0248	0.0218	0.0388	0.0854	0.0285
$P_{0.05}$	0.0558	0.0728	0.0358	0.1644	0.0548
$P_{0.10}$	0.0608	0.0668	0.0538	0.1814	0.0605
$P_{0.20}$	0.0768	0.0748	0.0838	0.2354	0.0785
$T_{0.05}$	0.0378	0.0258	0.0588	0.1224	0.0408
$T_{0.10}$	0.0458	0.0388	0.0488	0.1334	0.0445
$T_{0.20}$	0.0368	0.0678	0.0508	0.1554	0.0518
Total				1.1686	

Table 10. ANOVA table for the ovarian growth

Source of variation	Degrees of freedom	Sum of squares	Mean S.S.	F
Treatment	8	0.0107	0.0013	6.5 *
Error	18	0.0028	0.0002	
Total	26	0.0135		

* Differ significantly at 5 % level.

The critical difference for the pairwise of ovarian growth was 0.0177 at 5 % level.

Pairwise comparison of ovarian growth

$P_{0.20}$ 0.0781   *

$P_{0.10}$ 0.0601

$P_{0.05}$ 0.0544

$T_{0.20}$ 0.0514

$T_{0.10}$ 0.0441

$T_{0.05}$ 0.0404

$C_{0.20}$ 0.0281

$C_{0.05}$ 0.0184

$C_{0.10}$ 0.0111

Continous lines show no significant difference between treatments.
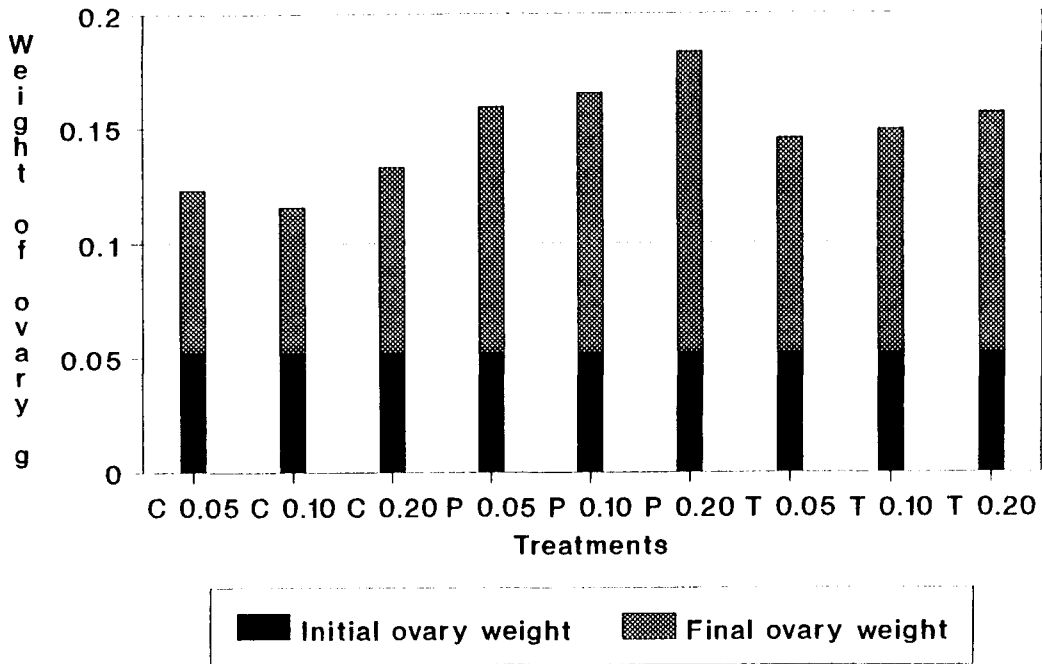
Fig. 17 Initial and final overy weights for different treatments
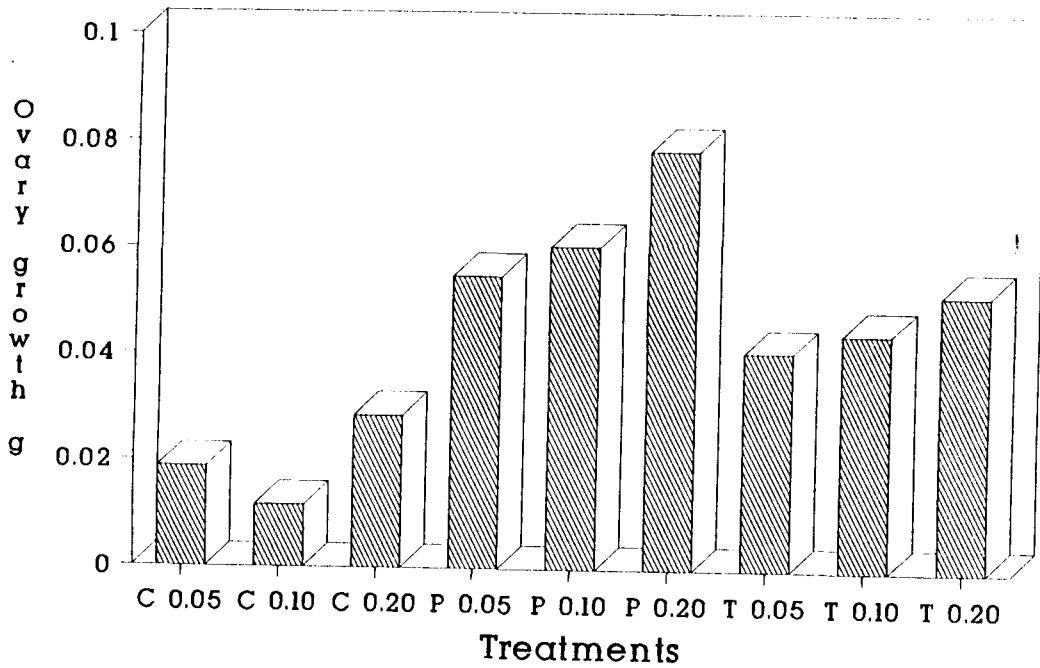
Fig. 18 Growth of overy for different treatments

Table 11. The calculated and table values for the comparison of
initial and final ovary weights

Treatments	Ovarian weight		't' value	
	Initial	Final	Calculated	Table
$C_{0.05}$	0.0522	0.1043	1.548	2.052
$C_{0.10}$	0.0522	0.0636	2.340	2.036
$C_{0.20}$	0.0522	0.0844	6.320	2.030
$P_{0.05}$	0.0522	0.1052	6.16	2.045
$P_{0.10}$	0.0522	0.1132	8.170	2.048
$P_{0.20}$	0.0522	0.1304	7.990	2.056
$T_{0.05}$	0.0522	0.0931	6.090	2.036
$T_{0.10}$	0.0522	0.0967	1.290	2.050
$T_{0.20}$	0.0522	0.1034	7.340	2.036

Table 12. Correlation of ovarian growth with body growth

Treatments	Body growth (g)	Ovarian growth (g)
$C_{0.05}$	1.07	0.0184
$C_{0.10}$	0.95	0.0111
$C_{0.20}$	0.66	0.0281
$P_{0.05}$	0.36	0.0544
$P_{0.10}$	0.28	0.0601
$P_{0.20}$	0.09	0.0781
$T_{0.05}$	0.61	0.0404
$T_{0.10}$	0.46	0.0441
$T_{0.20}$	0.32	0.0514

The correlation is calculated as - (0.9665).
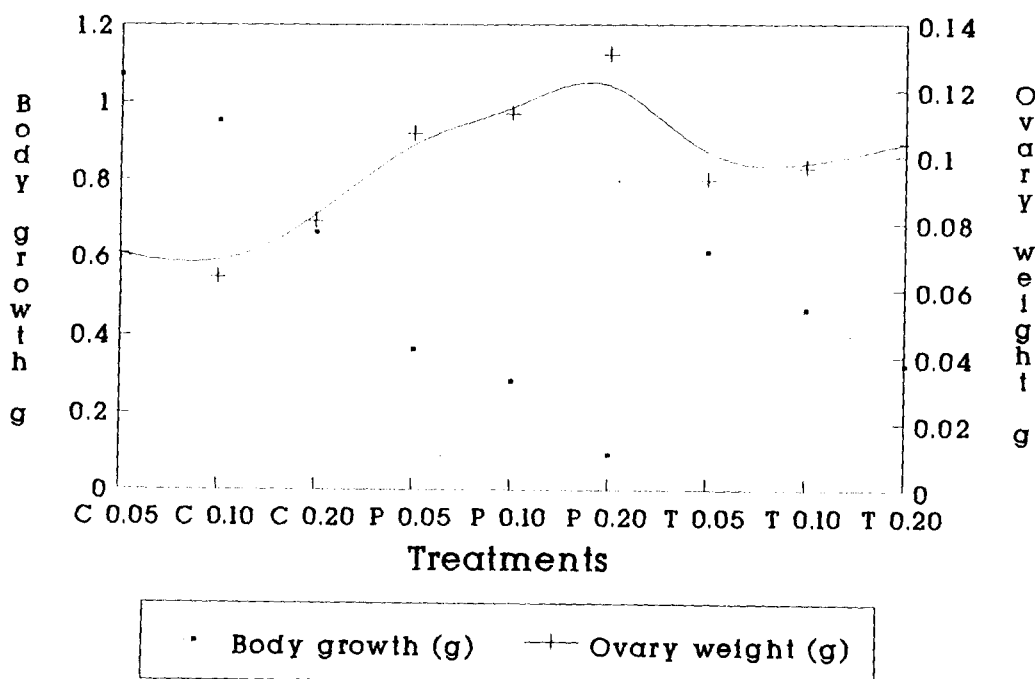
Fig. 19 Correlation between somatic growth and overian growth

V DISCUSSION

5.1 General conditions and procedure of experiment

Pond reared animals with hatchery produced seed were used to avoid interbrood variability. Only animals above 130 mm (TL) were used, as it was observed by Rao (1968) that the minimum size of maturity of *P. indicus* is 130.2 mm (TL) in the open waters. Though Muthu and Laxminarayana (1977) proposed a minimum size of 30 mm (CL) for the species to respond to ESA, many workers (Primavera *et al.*,1982; Makinouchi and Primavera,1987) have used very small specimens for successful maturation and spawning.

The experiment was done for 45 days so as to give the shrimps enough time for maturation and spawning. The period of April-June was selected to synchronize with the peak breeding activity of the Indian white shrimp in the wild (Panikkar and Menon,1956; George,1962; Subrahmanyam,1963; George *et al.*,1967).

Although very little is known of their action, it has been postulated that steroid hormones can stimulate vitellogenesis in crustaceans. The use of exogenous hormones to induce maturation of ovary and spawning is not well established.Further work is necessary to verify the specific role of these hormones in ovary maturation of Crustacea according to Yano (1985). Yano (1985,1987) based on his observations in *M.ensis* and *P. japonicus* deduced that progesterone may serve for ovarian vitellogenesis as a precursor of 17-a HP

stimulating Vg synthesis and/or release into haemolymph as the VSOH, as suggested by Junera *et al.* (1977) for the female amphipod, *O.gammarella*. Such a possibility is also discussed by Sarojini *et al.*(1985) based on their findings in *M.kistensis*. But, Nagabhushanam *et al.*(1980,1982) suspected that 17-a HP and 20-b HP bypass the synthesis of steroid mediator. The possible conversion of injected steroid hormone into desired hormone which may be naturally occurring in crustaceans is also speculated (Nagabhushanam *et al.*,1987; Sarojini *et al.*,1990).

Progesterone and related compounds have been detected in the reproductive organs of many crustacean species (Kanazawa and Teshima,1971; Teshima and Kanazawa,1971a,b,c,1973; Tcholakian and Eik-Nes,1971; Souty *et al.*,1982; Couch and Hagino,1983; Burns *et al.*,1984; Adiyodi,1985; Hazel,1986; Ollivier *et al.*, 1986; Couch *et al.*,1987; Tsukimura,1988; Fairs *et al.*,1989,1990; Quinitio *et al.*,1991; Young *et al.*, 1992) and these steroids have been found to induce ovary development in a number of prawns/shrimps (Kulkarni *et al.*,1979; Nagabhushanam *et al.*,1980,1982,1987; Joshi, 1980; Sarojini and Gyananath,1984; Sarojini *et al.*,1985;1987; Yano,1985; 1987; Chan and Lim,1988; Yashiro,1989; George and Khoo,1989; Tsukimura and Kamemoto,1991). In short, vertebrate steroid hormones seem to be present in crustacean tissues and exogenous application of these hormones produces effects consistent with a role promoting ovary maturation. It is likely that in future, the use and manipulation of steroid hormones in the regulation of penaeid maturation, is a real possibility (Quackenbush, 1991).

It was therefore decided to test the claim that progesterone and related compounds stimulate ovary development and spawning of *P. indicus* by progesterone treatment. Yano (1985) used 0.1 ug /g body weight (bw) to induce complete ovary maturation and spawning in *M. ensis* in a single course and noticed high mortality by administering high dosage and repeated injections, following the method of Kulkarni *et al.* (1979) in *Parapenaeopsis hardwickii*. He proposed species specific difference in progesterone response. So in the present experiment a lower dose (0.05 ug/g bw) and a higher dose (0.2 ug/g bw) were tested in addition to the effective dose of 0.1 ug/g bw tested for the metapenaeid by Yano (1985).

Since HUFA are essential for penaeids (Kanazawa,1985), it is suggested to include vitamin E also, as it prevents autoxidation of fatty acids (NRC,1983). Some of the recent researches point to the fact that vitamin E is important for quality of eggs in penaeids and scientists suspect some role for vitamin E in embryogenesis (Castillo *et al.*, 1989; Cahu and Fakhfakh,1990; Fakhfakh and Cahu,1990; Cahu *et al.*,1991; Fakhfakh *et al.*,1991; Alava *et al.*,1993b). Some Japanese scientists apparently had success with *P.japonicus* using diets containing high levels of vitamin E, upto 3 % of the diet (Dr.David Hewitt,Personal communication). So to test its effectiveness in the promotion of ovary maturation in *P.indicus*, vitamin E was included in one of the treatments in combination with progesterone.

The tanks were maintained in a separate room in order to minimize disturbance and also to simulate the natural conditions where the shrimps are known to breed. In this context it is to be noted that CMFRI (1994) had successfully induced ovary maturation and spawning of *P.indicus* under conditions of reduced light intensity without resorting to ESA. Since many tropical species gave good maturation response at a photoperiod of 16L:8D (Simon,1982; Primavera,1985; Nagabhushanam and Joshi,1986), the same day length was provided in the present experiment.

The inside walls of tanks were painted black, since Emmerson (1980) reported that there is more ovary maturation rate for *P.indicus* females maintained in black walled tanks. The injuries caused by the collision of the shrimps *P.setiferus* and *P.stylirostris* against the walls of the tanks were minimized by using walls painted black (Brown *et al.*, 1979,1980). Browdy and Samocha (1985) also used tanks painted black with white background for successful maturation and spawning of *P.semisulcatus*.

The water quality parameters during the experiment were within the acceptable level prescribed for maturation of penaeids by Primavera (1985). The findings of Laubier-Bonichon (1978), Lumare (1981) and Yano (1984) in *P.japonicus* and Beard and Wickins (1980) in *P.monodon* indicate that the effect of GSH or GIH on induction or inhibition of vitellogenesis in penaeid shrimps is controlled by temperature and/or photoperiod. As such, Yano (1987) advised to maintain the shrimps in a tank under optimal temperature and photoperiod when ovary maturation is to be induced by steroid

hormone treatment. Maturation of *P.indicus* was reported from captive rearing facilities (George,1974; Primavera and Yap,1979; Aquacop,1983; Bray and Lawrence,1992; Seat and Carlos,1994) and early maturation of the species reportedly takes place in brackishwater areas (Krishnamurthy and Ganapathy,1985). Since many workers have pointed out that salinity is not a constraint in ovary maturation of *Penaeus* spp. (Halder, 1978,1980; Opinano, cited by Primavera,1985; Posadas,1986; Ogle,1992a,b; Thompson, cited by Ogle,1992a) and even in species which are difficult to breed in captivity like *P.monodon*, (Primavera,1985) maturation of ovary was effectively induced by scientists like Halder (1978,1980) and Posadas (1986), a salinity of 25 ppt. was selected for the experiment.

5.2 Survival rate

There was no mortality due to the stress from injection of external materials. No mortality and allergic or abnormal response were reported for shrimps treated with hormone by previous workers (Nagabhushanam *et al.*,1982,1987; Koskela *et al.*,(1992), except Yano (1985) who experienced heavy mortality of *M.ensis* injected at high dose or when repeated injections done. The mortality rates which he had reported were 26.7 % and 12 % and 33.3 % and 0-30 % respectively for control and treated *M.ensis* after 31 days (Yano,1985) and *P. esculentus* after 35 days (Koskela *et al.*,1992). In the present study average survival rate was 88 % for control and 90 % for treated shrimps, after an

experimental period of 45 days, indicating that the conditions provided during the course of study were quite satisfactory. There was no significant difference in survival between the control and treatments. But by performing ESA, heavy mortality was reported (Primavera,1985; Bray and Lawrence,1992). Thus the occurrence of heavy mortality in ESA technique would warrant the development of some other method like the hormonal injection, which may help in lowering the mortality rate of the valuable broodstock.

5.3 Visual assessment of gonadal development

One of the most effective methods of studying reproductive state of the marine organisms is to determine the degree of gonadal growth (Castille and Lawrence,1991). In penaeid shrimp, gonadal growth can be evaluated using a number of parameters. For females the common method has been visual assessment of the ovaries on the basis of egg size and colour (King,1948; Cummings,1961; Subrahmanyam,1965; Tuma,1967; Rao,1968; Villaluz *et al*.,1979; Primavera,1980; Motoh,1981; Crocos and Kerr,1983; Yano,1985; Tan-Fermin and Pudadera,1989). In the present experiment there was no sign of ovary development clearly visible through the dorsal integument. Only Yano (1985) has reported externally visible maturation stage differences and spawning as a result of progesterone injection in *M.ensis*. He found ovarian stages III and II in 4 and 2 animals respectively out of the 15 test shrimps and stage II in 7 out of 15 control shrimps. Thus only 27 % and 17 % of treated metapenaeid shrimps matured and spawned respectively in

response to progesterone treatment. No other study so far recorded any visible change in the stages of ovary on external viewing by the application of progesterone. In the present study, on a closer examination of the dissected ovary, it was found that they have become larger in size and have become transluscent compared to the thin and transparent nature in the beginning of the experiment; progesterone alone and combination of progesterone and vitamin E treated shrimp ovaries were found to be distinctly different from those of untreated controls.

5.4 Gonado somatic index (GSI)

The simplest indicator of the reproductive state of the marine organisms is the GSI (Grant and Tylor,1983). In this method of assessing gonadal growth, changes in the ratio between gonad weight and body weight is taken into account. This has been variously used in penaeids (Lawrence et al.,1979; Joshi,1980; Lawrence and Castille,1991). Since there was no change in external appearance of ovaries when viewed through the dorsal integument, the GSI was used for a comparison of the effect of treatments on the ovary maturation of the shrimp. GSI values of shrimps treated with progesterone and a combination of progesterone and tocopherol, except P+T at 0.05 ug/g bw, grouped together from the control shrimps indicated that some sort of impetus is there for penaeid shrimp ovary to develop in response to progesterone and vitamin E.

In experiments involving progesterone treatment the only report of difference in GSI was furnished by Joshi (1980) in *P.stylifera*. Chan and Lim (1988) failed to get any difference in GSI for progesterone treated *P.merguiensis* in comparison to the controls, although they could observe histological changes between these two. But Tsukimura and kamemoto (1991) failed to obtain any response from *P.vannamei* ovaries *in vitro* by the treatment of progesterone. Anon. (1992) failed to effectively enhance ovary development in preadult *P.monodon* using the 17-a HP and 17-b estradiol. Koskela *et al.* (1992) also got no maturation of *P.esculentus* ovary either in terms of GSI or histological examination in response to prostaglandins, 17-a HP and 17-b estradiol treatment singly or in combination and as such they strongly question the claim that the steroid hormones act as VSOH. But in the present study, the significant difference in GSI for progesterone and progesterone and tocopherol treatments, points to the fact that they may have some role in contributing to the ovarian development in *P.indicus*.

5.5 Ovarian growth (OG)

The difference between the initial and final weights of ovary subjected to analysis using 't' test had shown that the pattern is the same as that of the GSI data analysis. It can be found that the growth of ovary increased with increase in progesterone in the case of progesterone treatment and with progesterone and tocopherol in the progesterone-vitamin E combination treatment, whereas it was minimum for the control shrimps. This

observation supports further the role of progesterone and vitamin E in initiating ovarian growth in penaeid shrimps. In the present study, although there was no clearly distinguishable change in the ovarian stage, GSI and ovarian growth were higher for shrimps treated with progesterone alone and in combination with tocopherol (especially at higher dosages) than the control shrimps. This positive response to progesterone and tocopherol by shrimps with the eyestalks intact, may be through stimulation of GSH or by way of accelerating some process or metabolic pathway connected with vitellogenesis. The response of progesterone not being not so prominent may be because of the GIH still being produced by the ES. Similar positive response to hormonal treatment was reported by Bomirski and Klek-Kawinska (1976) using HCG and Zukowska-Arendarczyk (1981) by using FSH and LH, on the vitellogenesis in *C.crangon*, irrespective of the presence of endogenous GIH, although they have failed to record any external change in ovary maturation. Koskela *et al.* (1992) explained that the failure to bring about ovary development in *P.esculentus* by hormone treatment was partly due to the endogenous production of GIH. Eastman-Reks and Fingerman (1984) have speculated that GIH from ES may inhibit the synthesis of GSH. Yano (1992a) agreeing to this statement, has pointed out that in penaeid shrimp, GSH levels may increase further with advancement of vitellogenesis, parallel to a decrease in the level of GIH. So it is possible that GIH has got a profound influence on the gonadal growth of penaeid shrimps and its removal allows the synthesis of Vg in significant proportions as observed by Adiyodi (1985),

Charniaux-Cotton (1985), Fingerman (1987), Keeley (1991), Mohamed and Diwan (1991), Quackenbush (1991) and Dr.David Hewitt (Pers. comm.). The reduction in GIH due to ESA may help the GSH to function fully (Babu *et al.*,1980; Mohamed and Diwan,1991; Yano,1992a) and to complete the ovarian maturation in crustaceans. So it can be reasonably suspected that the circulating GIH would have prevented full maturation of *P.indicus* ovary. It can be stated that GIH has the say while the female shrimps are in the sexually quiescent state. As stated by Quackenbush (1991) the GIH not only affects the ovarian maturation, but also the steroid endocrine centres as well. In the present experiment, although progesterone would have contributed to vitellogenesis, it would not have been sufficient to overcome the suppressive action of GIH to make the shrimp complete the ovarian maturation and spawn. Another reason for not obtaining full maturation may be the low titre/absence or non-functioning of GSH due to inactivation by GIH, since it is generally accepted that the ovarian maturation in crustaceans is regulated by the two antagonistic neurohormones, the GIH from XO-SG neuroendocrine complex in ES and the GSH from brain and TG (Adiyodi and Adiyodi,1970; Yano,1992a).

Dr. David Hewitt (Pers.comm.) finds little convincing information on the effect of steroid hormone and he feels that GIH, JH and MF all have a higher level of action on the ovarian maturation of shrimps compared to steroids. Thus from the present investigation it may be assumed that steroid hormones including progesterone are not capable on their own to induce full ovary maturation and spawning in penaeid shrimps. So it can be

expected that a GIH suppressor (Keeley,1991; Dr.David Hewitt, pers.comm.) may also be necessary, besides the ovary maturation inducers like steroid hormones, JH and MF for inducing full ovarian maturation and spawning in penaeid shrimps.

It was found that except for the treatments $C_{0.05}$ and $T_{0.10}$ there existed significant difference between the initial gonadal weight and final gonadal weight, indicating that there is some growth of ovary in shrimps irrespective of the injection material. This points to the fact that the experimental conditions and water quality parameters maintained in tanks were sufficient to initiate and/or maintain maturation of ovary in *P.indicus*. Such an observation of good maturation conditions provided in tanks promoting ovary growth is also reported by Koskela *et al.* (1992) from their findings that 4 shrimps including 2 control shrimps progressed to higher maturation stages compared to the hormone treated *P.esculentus*.

It is gratifying to note that attempts are being made in different parts of the world (O'Sullivan,1994; Walker,1994), to find an alternative to the present method of ESA. But till then, the present method of ESA will have to be relied upon with all its demerits, to achieve successful spawning of shrimps in captivity.

5.6 Somatic growth (SG)

The PSG for control groups was generally significantly higher than those of shrimps treated with progesterone and P+T. The critical difference analysis showed that

treatments $P_{0.2}$ and $P_{0.1}$ are having exceptionally low PSG compared to rest of the treatments. The control values were grouped together with $T_{0.05}$ to form a class of high PSG, indicating that while progesterone and tocopherol treatments contributed to growth of ovary, the somatic growth was affected. But, Koskela et al.(1992) found no difference in SG or OG by hormone treatment and others noted high growth rate (GR) for ESA shrimps (Kelemec and Smith,1980; Choy,1987) and prawns (Ponnuchamy et al.,1981; Pandian and Sindukumari,1985; Kumari and Pandian,1987; Murugadass et al.,1988; Chakravarthy,1992). While some scientists reported higher GR for non-ablated penaeids (Chamberlain et al.,1981a; Primavera et al.,1982; Browdy et al.,1986; Hansford et al.,1993), others noted no difference between the stalked and destalked penaeids and palaemonids (Emmerson,1983; Browdy and Samocha,1985; Bijulal,1994). The extreme cases of increased growth rate for BESA animals compared to unablated animals has also been recorded by Alikunhi et al.(1975) and Chakravarthy (1992). The decreased growth rate in the hormone treated shrimps may be attributed to the allocation of energy reserves to ovary development rather than to somatic growth.

5.7 Correlation between somatic growth and ovarian growth

In the present investigation ovary growth was found to be strongly correlated reversely with somatic growth. For gonad maturation to proceed in crustaceans, production of GIH and its titres within the body, must be low or non-existent, while production of MIH must be high so that moulting process does not compete for metabolic reserves

(Adiyodi and Adiyodi,1970). Adiyodi (1980) opines that growth as well as reproduction need sizeable amounts of energy and total expendable energy in any organism at any point of time is finite; it is mandatory that the animal resorts to only one physiologically expensive process at a time, either growth or reproduction. Many other scientists agree to this school of thought that most crustaceans especially females have to cope, in the adult state with high energy demanding processes of SG and ovary development and as such these two processes biologically programmed antagonistically (Bliss,1966; Aiken and Waddy,1976; Quackenbush and Herrnkind,1981; Adiyodi and Subramoniam,1983; Adiyodi,1985; Charniaux-Cotton,1985; Charniaux-Cotton and Payen,1988; Nelson,1991; Chang,1992). But Quackenbush (1986) hypothesizes that crustaceans regulate ovary growth and SG to achieve an optimum balance between these two and Emmerson (1983) proposes that energy has to be directed to growth in addition to reproduction so that one process does not interfere with the other. Bray and Lawrence (1992) are of the view that in nature an organism would not be expected to develop eggs constituting some 10 % of female body weight, unless nutrients are available for first metabolism, second growth and lastly reproduction. Observations in the wild *P.indicus* (Read and Caulton,1980), *P.merguiensis* (Crocos and Kerr,1983) and *P.semisulcatus* (Schlagman *et al.*,1986) support this concept. For wild *P.indicus* and probably for other species of penaeids synergistic occurrence of ovary growth and SG may be there, but in captive females balancing of these processes depend on controlling factors such as nutrition, temperature, light quality

and intensity, photoperiod and container size (Emmerson,1980). Moreover, Emmerson *et al*. (1983) and Hansford *et al*. (1993) anticipate that by providing good nutrition these two functions can be made synergistic. But Primavera and associates (1982) noted high growth rate for unablated *P.indicus* compared to ablated females; though there was no significant difference between the treatments. Similar results of high SG for unablated shrimp were also recorded by other scientists (Emmerson,1980; Browdy *et al*.,1986; Choy,1987; Hansford *et al*.,1993). But Wickins and Beard (1974) found no retardation of growth in *M.rosenbergii* by egg production and they assume that the fundamental difference between caridean and penaeid reproduction is the difference in response to ovary growth; in the latter more egg being produced within a short time span resulting in a decreased growth rate in reproducing females. Although shrimps are placed in crustacean group II in which SG and ovary growth takes place simultaneously by Adiyodi (1985) and Charniaux-Cotton (1985), Quackenbush (1986) and Mohamed and Diwan (1991) like them to be included in between group I (reproduction and growth being antagonistic) and group II. Mohamed and Diwan (1991) comparing their results of UESA and BESA propose that emphasis during BESA (all inhibiting hormones removed) is towards moulting and during UESA (half of the inhibiting hormone removed) it is towards maturation. They state that GIH and MIH are the same, agreeing to the view of Adiyodi and Adiyodi (1970) that MIH and GIH are related molecules. But this claim is contradictory to the finding of Quackenbush (1986) that these two are different peptides.

The present observations in *P.indicus* supports the view that the two energy demanding

processes, ovary growth and SG, are to a certain extent antagonistic in nature.

VI SUMMARY

1. Many negative effects like high mortality rate, low fecundity, low egg viability, weak offspring and short span of productive life for brooders experienced as a result of eyestalk ablation (ESA) necessitated scientists to develop alternative ways to this generally accepted method of inducing ovarian maturation in penaeid shrimps.

2. The efficacy of progesterone and a combination of progesterone and tocopherol in inducing ovarian maturation of pond-reared *Penaeus indicus* under controlled conditions was investigated.

3. Progesterone and tocopherol were dissolved separately in ethanol and three test concentrations (0.05ug, 0.1ug and 0.2ug/g body weight -bw) were prepared.

4. The progesterone was dissolved in ethyl alcohol and made upto the desired concentrations by dilution with ethyl alcohol. Tocopherol was also dissolved in ethanol and to prepare the mixture, they were mixed in 7:3 (V/V) ratio to get 3 different test concentrations as above. Ethyl alcohol at the respective dosages served as the control treatment. Administration of these materials was done using a micro-litre syringe through the first abdominal segment of the shrimp.

5. It was found that the survival of shrimp was not affected much by treatment with these exogenous agents. The conditions provided for the study seems to be quite satisfactory which is reflected in the high level of survival in both the control (88 %) and the test treatments (90 %).

6. No visible change in the maturation stage of the ovary could be observed through the dorsal integument of the animal. But, on a closer examination after dissecting out the ovary, increase in size and change of ovary were noticed which were more pronounced in the case of the hormone and a combination of hormone and vitamin treated shrimps.

7. The gonado somatic index data analyzed by ANOVA technique revealed that all the treatments which received progesterone, and those which received combination of progesterone and tocopherol except at the low dose of 0.05 ug/g bw, showed significantly higher GSI compared to the alcohol-treated shrimps; the progesterone treatment at 0.2 ug and 0.1ug/g bw giving the best results.

8. Ovarian growth analysis also gave similar trend as the GSI value analysis. All the treatments except the control at 0.05 ul/g bw and combination treatment at 0.1ug/g bw levels gave significant difference in ovarian growth.

9. Percentage somatic growth for control groups registered higher growth rate whereas lowest values were observed for progesterone treatments at 0.2 ug and 0.1 ug/g bw.

10. Correlation of ovarian growth with that of somatic growth gave a strong inverse relationship between gonadal growth and somatic growth, indicating antagonism between these two phenomena.

11. The presence of endogenous gonad inhibiting hormone would have hindered the development of ovary in this penaeid shrimp beyond a certain point. So this study reveals that complete development of ovary and spawning of shrimp cannot be achieved by the mere application of progesterone or tocopherol, in the presence of endogenous GIH from the eyestalk.

12. It is possible that steroid hormones like progesterone may bring about full maturation of penaeid ovary, if they are administered alongwith a GIH suppressor.

VII REFERENCES

Achuthankutty,C.T. and Parulekar,A.H.(1986) Growth of penaeid prawns in Goa waters.*Indian J.Mar.Sci.*,**15**:117-120.

Adiyodi,K.G.(1980) Some Thoughts on the Evolution of "Reproductive" Hormones in Invertebrates. In:T.Subramoniam and S.Varadarajan (eds.), *Progr.Invert.Reprod. Aquacult.*, New Century Printers, Madras.pp.89-93.

* Adiyodi,R.G.(1968) On reproduction and moulting in the crab, *Paratelphusa hydrodromous*. *Physiol.Zool.*,**41**:204-209.

*Adiyodi,R.G.(1969) Insect Hormones other than pheromones. *Vijn. Kair*, **1**:57-65.

Adiyodi,R.G.(1978) Endocrine control of ovarian function in crustaceans. In:P.J.Gaillard and H.H.Boer (eds.), *Comparative Endocrinology*. Elsevier North Holland Biomedical Press, Amsterdam, pp.25-28.

Adiyodi,R.G.(1985) Reproduction and Its Control. In:D.E.Bliss and L.H.Mantel (eds.), *The Biology of Crustacea*. Academic Press, New York. **IX**:147-215.

Adiyodi,K.G. and Adiyodi,R.G.(1970) Endocrine Control of Reproduction in Crustacea. *Biol.Rev.*,**45**:121-165.

Adiyodi,R.G. and Subramoniam,T.(1983) Arthropods-Crustacea. In: K.G. Adiyodi and R.G.Adiyodi (eds.), *Reproductive Biology of Invertebrates*,Academic Press,New York.**1**:443-495.

*Aiken,D.E.(1969) Photoperiod, endocrinology and the crustacean molt cycle. *Science*, **164**:149-155.

Aiken,D.E. and Waddy,S.L.(1976) Controlling growth and reproduction in the American lobster. *Proc.World Maricult.Soc.*,**7**: 415-430.

*Alava,R.A.(1979) Effect of light quality and ablation on ovarian maturation in pond reared *Penaeus monodon* Fabricius.*M.S. Thesis*, Univ.Philippines, Legenes,Iloilo, 75pp.

Alava,R.V.,Kanazawa,A.,Teshima,S.and   Koshio,S.(1993a)   Effect   of   Dietary Phospholipids and n-3 Highly Unsaturated Fatty Acids on Ovarian Development of kuruma prawn. *Nipp.Suis. Gakk.*, **59**: 345-351.

Alava,R.V.,Kanazawa,A.,Teshima,S. and Koshio,S. (1993b) Effects of Dietary Vitamins A,E and C on the Ovarian Development of *Penaeus japonicus. Nipp. Sus.Gakk.*, **59**:1235-1241.

Alava,V.R.,Kanazawa,A.,Teshima,S. and Koshio,S.(1993c) Effect of L-Ascorbyl - 2-Phosphate Magnesium on Gonadal Maturation of *Penaeus japonicus. Nipp.Suis. Gakk.*,**59**:691-696.

*Alikunhi,K.H.,Poernomo,A.,Adisukreno,S.,Bodino,M.   and   Busman,S.   (1975) Preliminary observations on induction of maturity and spawning in *Penaeus monodon* Fabricius and *Penaeus merguiensis* de Man by eyestalk extirpation. *Bull.Shri.Cult.Res .Cent.*,**1**:1-11.

*Amaldoss,G.,Hsue,N.-C.,Kulkarni,G.K. and Fingerman,M. (1991) Effect of unilateral eyestalk ablation on the ovary of *Macrobrachium rosenbergii* and *Penaeus monodon*: A Comparative study. In:R.N.Saxena, K.Muralidhar, L.Bhagat, N.Seghal, T.Saxena and P.Kaushed(eds.),*Curr.Themes Comp.Endocrin.*, Delhi Univ.Press, New Delhi,pp.117-118.

*Anilkumar,G.(1980) Reproductive Physiology of female crustaceans. *Ph.D.Thesis.* Calicut Univ.,India.

Anilkumar,G. and Adiyodi,K.G.(1981) Ovarian Growth Induced by Eyestalk Ablation during the Prebreeding season is not normal in the crab, *Paratelphusa hydrodromous* (Herbst). *Inter.J. Invert.Reprod.*,**2**:95-105.

Anonymous (1992) Improved Broodstock Maturation Techniques for Black Tiger Shrimp. *Asian Shrimp News*, ASSC **3**:11.

* Aoto,T. and Nishida,H.(1956) Effect of removal of the eyestalks on the growth and maturation of oocytes in a hermaphroditic prawn, *Pandalus kessleri. J.Fac. Sci. Hokkaido Univ.(ser.VI)*, **12**:412-424.

Aquacop (1975) Maturation and Spawning in Captivity of Penaeid Shrimp: *Penaeus merguiensis* de Man, *Penaeus japonicus* Bate, *Penaeus aztecus* Ives,*Metapenaeus ensis* de Haan and *Penaeus semisulcatus* de Haan. *Proc.World Maricult.Soc.*, **6**:123-132.

Aquacop (1977a) Reproduction in Captivity and Growth of *Penaeus monodon*, Fabricius in Polynesia. *Proc. World Maricult. Soc.*, **8**: 927-945.

*Aquacop (1977b) Observations on the maturation and reproduction of penaeid shrimp in captivity in a tropical medium. *Aquacult. Workshop*,ICES, 10-13,May,1977, Brest, France, 34pp.

Aquacop (1979) Penaeid Reared Broodstock: Closing the Cycle of *P.monodon, P. stylirostris* and *P. vannamei.Proc. World Maricult. Soc.*,**10**:445-452.

Aquacop (1982) Reared Broodstock of *Penaeus monodon*. *Proc.Symp. Coastal Aquaculture*. Mar.Biol.Assoc.India,**I**:55-62.

Aquacop (1983) Constitution of Broodstock, Maturation, Spawning and Hatchery Systems for Penaeid Shrimps in the Centre Oceanologique du Pacific. In: J.P.McVey (ed.), *Hand Book on Mariculture,Crustacean Aquaculture*. CRC Press,London, **I**: 105-121.

Arnstein,D.R. and Beard,T.W.(1975) Induced Maturation of the Prawn *Penaeus orientalis* Kishinouye in the Laboratory by means Eyestalk Removal. *Aquaculture*, 5:411-412.

*Arvy,L.,Echalier,G. and Gabe,M.(1956) Organe et gonade chez *Carcinus maenas*L. *Ann.Sci.nat.zool.biol.animale*,**18**:263-267.

*Babu,D.E.,Shyamasundari,K.and Rao,K.H.(1980) Correlative changes in the neurosecretion and ovarian growth after bilateral ablation of eyestalk in the crab, *Menippe rumhii*. *Indian J. Exp.Biol.*,**18**:265-268.

*Barber,S.B.(1961) Chemoreception and thermoreception. In:T.H. Waterman (ed.),*The Physiology of Crustacea*.Academic Press, New York.II:109-131.

Beard,J.W.,Wickins,J.F.and Arnstein,D.R.(1977) The Breeding and Growth of *Penaeus merguiensis* de Man in Laboratory Recirculation Systems. *Aquaculture*, 10: 275-289.

Beard,J.W. and Wickins,J.F. (1980) Breeding of *Penaeus monodon* Fabricius in Laboratory Recirculation Systems. *Aquaculture*, **20**:79-89.

*Bell.T.A. and Lightner,D.V. (1988) *A handbook of normal penaeid shrimp histology*. World Aquacult.Soc.,Los Angeles.114pp.

*Beltz,B.S.(1988) Crustacean neurohormones.In: H.Laufer and D. Downer (eds.), *Endocrinology of selected invertebrate types.* Alan R.Liss,New York, Inv. Endocrinol.,III:235-258.

*Berreur-Bonnenfant,J. and Lawrence,F.(1984) Comparative effect of farnesylacetone on macromolecular synthesis in gonads of crustaceans. *Gen.Comp. Endocrinol.*, 54: 462-468.

Bijulal,P.S.(1994) Effect of eyestalk ablation on growth and reproduction of *Macrobrachium equidens* Dana*M.F.Sc.Thesis,* Kerala Agricultural University, Thrissur. 121pp.

*Bliss,D.E.(1966) Physiological process and neurosecretion as related to ecdysis and reproduction: Relationship between reproduction and growth in decapod crustaceans. *American Zool.,*6:231-233.

Bomirski,A. and Klek,E. (1974) Action of Eyestalk in the Ovary of *Rhithopanopeus harrissi* and *Crangon crangon* (Crustacea: Decapoda). *Mar.Biol.,*24:329-337.

*Bomirski,A.and Klek-Kawinska,E.K.(1976) Stimulation of oogenesis in the sand shrimp, *Crangon crangon,* by a human gonadotropin.*Gen.Comp.Endocrinol.,*30: 239-242.

*Borst,D.W.,Laufer,H.,Landau,M.,Chang,E.S.,Hertz,W.A.,Baker,F.C.andScooley, D.A. (1987) Methyl fernesoate and its role in Crustacean Reproduction and Development. *Insect Biochem.,* 17: 1123-1127.

*Bouchan,A.G. and Fontaine,M.T.(1984) Chemoreception et comportement de reproduction chez les crustaces.*Oceanis,*10:151-168.

Bouchon,D.(1991) Biological Clock in Seasonal Reproductive Cycle in the ditch shrimp *Palaemonetes varians* Leach.II.Ovarian State-dependent Response to Non-dial-light-dark Cycles. *J.Mar.Biol. Ecol.,*146:13-26.

Bouchon,D., Souty- Grosset,C., Mocquard, J., Chentoufi, A. and Juchault,P.(1992) Photoperiod and Seasonal Breeding in Aquatic and Terrestrial Eumalacostraca. *Invert.Reprod.Dev.,*22:203- 212.

Bray,W.A. and Lawrence,A.L.(1984) Sourcing *Penaeus setiferus*: a summary of larval production,incidence of capture of mated females and mating incidence by time of the day on research cruises,1981-1983. *J.World Maricult.Soc..,*4:11-28.

Bray,W.A. and Lawrence,A.L.(1988) Influence of dietary fattyacids on reproduction of *Penaeus stylirostris. J. world Aquacult.Soc.*, **19**:18A (Abstract only).

Bray,W.A.and Lawrence,A.L.(1992) Reproduction of *Penaeus* species in captivity. In: W.A.Fast and L.J.Lester (eds.), *Marine shrimp culture: Principles and practices.* Elsevier Science Publishers, Amsterdam. pp.93-169.

Bray,W.A., Lawrence,A.L. and Lester,L.J. (1990) Reproduction of *Penaeus stylirostris* fed various levels of Total Dietary Lipid. *J.World Maricult.Soc.*,**21**:41-52.

Bray,W.A,Lawrence,A.L.and Leung-Trujillo,J.K.(1989) Reproductive Performance of Ablated *Penaeus stylirostris* fed Soylecithin Supplement. *J.World Maricult.Soc.*, **20**:19A (Abstract only).

Brisson,S.(1986) Observations on the courtship of *Penaeus brasiliensis. Aquaculture*,**53**: 75-78.

*Browdy,C.L.(1989) Aspects of reproductive biology of *Penaeus semisulcatus* de Haan (Crustacea DecapodaPenaeidae).*Ph.DDissertation.*Tel Aviv Univ.,TelAviv. 138pp.

Browdy,C.L. and Samocha,T.M.(1985) The Effect of Eyestalk Ablation on Spawning, Molting and Mating of *Penaeus semisulcatus* de Haan. *Aquaculture*,**49**:19-29.

Browdy,C.L., Hadani,A., Samocha,T.M. and Loya,Y. (1986) The Reproductive Performance of Wild and Pond Reared *Penaeus semisulcatus* de Haan. *Aquaculture.*, **59**:251-258.

*Browdy,C.L., Hadani,A., Samocha,T.M. and Loya,Y. (1989) An evaluation of frozen *Artemia* as a dietary supplement for the stimulation of reproduction in penaeid shrimp. In:N. Depauw, E.Jaspers,H.Ackeford and N.Wilkins(eds.), *Aquacult.-A biotechn.progr.*.Europ.Aquacult.Soc.,Bredene.pp.617-623.

Brown,A.,Jr.,McVey,J.,Middleditch.,B.S. and Lawrence,A.L.(1979) Maturation of white shrimp (*Penaeus setiferus*) in Captivity. *Proc.World Maricult.Soc.*,**10**:435-444.

Brown,A.,Jr.,McVey,J.P,Scott,B.M.,Williams,J.D.,Middleditch,B.S. and Lawrence,A.L. (1980) The Maturation and Spawning of *Penaeus stylirostris*under Controlled Conditions.*Proc.World Maricult.Soc.*,**11**:488-499.

*Brown,F.A.,Jr.and Jones,G.W.(1949) Ovarian inhibition by Sinus- gland principle in fiddler crab. *Biol.Bull.*,**91**:228-232.

Bueno,S.I.D.S.(1990) Maturation and Spawning of the white shrimp *Penaeus schmitti* Burkenroad,1936,under large Scale Rearing Conditions. *J.World Aquacult. Soc.*, **21**:170-179.

Burns,B.G.,Sangalang,G.B.,Freeman,H.C.and McManeniy,M.(1984) Isolation and identification of testosterone from the serum and testes of the American lobster (*Homarus americanus*). *Gen. Comp.Endocrinol.*,**54**:429-432.

Byard,E.H.and Aiken,D.E.(1984) The relationship between molting, maturation and a hemolymph female-specific protein in the lobster, *Homarus americanus. Comp. Biochem.Physiol.*,**77(A)**:749-757.

*Byard,E.H,Shivers,R.K.and Aiken,D.E.(1975) The mandibular organ of the lobster, *Homarus americanus. Cell Tiss.Res.*,**162**: 13-22.

Cahu,C. and Fakhfakh,M.(1990) Effect of dietary vitamin E on reproduction of penaeid shrimps. *I.Zootech.Rep.*, World Aquacult.Soc.Annu.Conf. p.7 (Abstract only).

*Cahu,C.,Fakhfakh,M. and Quazugual,P.(1991) Effect of dietary a- tocopherol levels on reproduction of *Penaeus indicus.* In:P.Lavens,P.Sorgeloos,E.Jaspers and F. Ollevier (eds.), *Larvi - 91.Fish and Crust.Larvicult.Symp.* European.Aquacult. Soc., Bredene.pp.242-244.

*Cahu,C.,Fauvel,C. and Aquacop (1986) Effects of food fatty acid composition of *Penaeus vannamei* broodstock on egg quality. *ICES Maricult.Comm.CM.*,**1986F**: 29.
Cahu,C.,Guillaume,J.C.,Ste'phan,G. and Lim,L.(1987) Essentiality of phospholipid and highly unsaturated fatty acid in *Penaeus vannamei* fed purified diets. *World Aquacult.Soc. 18 th Ann. Conf.*, p.22 (Abstract only).

*Cahu,C.,Se'v'ere,A. and Quazugual,P.(1988) Variation of lipid composition in *Penaeus indicus* during larval development. *ICES Maricult.Comm.*,**1988/F22**:11.

*Cahu,C.,Villete,M,Quazugual,P.and Guillaume,J.(1993) The effect of n-3 highly unsaturated fatty acid and vitamin E supplement in broodstock feed on reproduction of *Penaeus indicus.* In: S.Kaushik (Ed.), *Fish Nutr.Practice.Proc.IV th Intern. Symp. Fish Nutr.Feeding.*Biaritz,France. pp.589-598.

Cahu,C.,Guillaume,J.C.,Ste'phan,G. and Chim,L.(1994) Influence of phospholipid and highly unsaturated fatty acids on spawning rate and egg and tissue composition in *Penaeus vannamei* fed semi-purified diets. *Aquaculture*,**126**:159-170.

Caillouet,A.C.,Jr.(1972) Ovarian maturation induced by eyestalk ablation in pink shrimp, *Penaeus duorarum* Burkenroad. *Proc. World Maricult.Soc.,3*:205-225.

*Carlilse,D.B.(1953) Studies on *Lysmata seticaudata* Risso (Crustacea Decapoda) V.The ovarian inhibiting hormone and the hormone inhibition of sex reversal. *Pubbl.staz. Zool. Napoli*, **24**:455-472.

*Carlilse,D.B.(1954) The X-organ-sinus gland complex, somatotropin,the ovarian inhibiting hormone and sex reversal in *Lysmata seticaudata* (Natantia Hippolysidae). *Pubbl.Staz.Zool. Napoli,*24 suppl.79.

Castille,F.L. and Lawrence,A.L.(1991) Reproductive Studies Concerning Natural Shrimp Populations : A Description of Changes in the Size and Biochemical Composition of the Gonads and Digestive Glands in Penaeid Shrimp. **In**: P.F.De Loach,W.J. Dougherty and M.A.Davidson (eds.), *Frontiers of Shrimp Research*. Elsevier Science Publishers,Amsterdam. pp.17-37.

*Castillo,M.,A.,Cahu,C. and Stephan,G.(1989) Alpha-Tocopherol level of Eggs and Organs of *Penaeus indicus*. *Biochem.Mar. Organisms.*15: 553-560.

*Chaix,J.-C. and De Reggi,M.(1982) Ecdysteroid levels during ovarian development and embryogenesis in the spider crab, *Acathonyx lunatus*. *Gen.Comp.Endocrinol.*, **47**: 7-14.

*Caubier,J.-L.,Lafon,R.,Rene,F. and Sales,C.(1979) Etude de la maturation et al ponte chez *Penaeus japonicus* in captivite. **In**: T.V.R.Pillay and W.Dill (eds), *Adv. Aquacult.*. Fishing News Books Ltd.,Surrey. pp.277-284.

Chakravarthy,M.S.(1992) Effect of eyestalk ablation on moulting and growth in prawn, *Macrobrachium rosenbergii*. *Indian J. Mar.Sci.*, **21**:287-289.

*Chamberlain,G.W.(1988) Stepwise investigation of environmental and nutritional requirements for reproduction of penaeid shrimp. *Ph.D. Dissertation,*Texas A & M Univ.,Texas. 210pp.

Chamberlain,G.W.and Garvais,N.F.(1984) Comparison of unilateral eyestalk ablation with environmental control for ovarian mat- uration of *Penaeus stylirostris.J.World Aquacult.Soc.,*15:29-30.

Chamberlain,G.W. and Lawrence,A.L.(1981a) Maturation, Reproduction and Growth of *Penaeus vannamei* and *P.stylirostris* fed Natural Diets. *J.World Maricult.Soc.*, **12**:209-224.

Chamberlain,G.W. and Lawrence,A.L.(1981b) Effect of light intensity and male and female eyestalk ablation on reproduction of *Penaeus stylirostris* and *P.vannamei*. *J.World Maricult.Soc.*, **12**:357-372.

*Champion,H.F.B.(1987) The functional anatomy of male reproductive system in *Penaeus indicus*. *South African J.Zool.*,**22**:297- 307.

Chang,C.,Lee,F.,Huang,Y. and Hong,T.(1994) Purification and Characterization of the female Specific Protein (Vitellogenin) in Mature female hemolymph of the prawn, *Penaeus monodon*. *Invert.Reprod.Dev.*,**25**:185-192.

*Chang,E.S.(1984) Ecdysteroids in Crustacea:Role in reproduction,molting and larval development.In:W.Angels,W.H.Clarke,A.Fisher,P.J.W.Olive and D.F.Went (eds.), *Adv.Inv.Reprod.*, Elsevier Science Publishers,Amsterdam,**III**:223-230.

Chang,E.S.(1991) Crustacean Moulting Hormones:Cellular Effects, role in Reproduction and Regulation by Molt-inhibiting Hormones. In: P.F.DeLoach, W.J.Dougherty and M.A.Davidson (eds.), *Frontiers of Shrimp Research*. Elsevier Science Publishers, Amsterdam. pp.83-106.

Chang,E.S.(1992) Endocrinology. In: W.A.Fast and L.J.Lester (eds.), *Marine shrimp culture:Principles and practices.*Elsevier Science Publishers,Amsterdam.pp.53-92.

Chang,E.S.,Hertz,W.A. and Prestwich,G.D.(1992) Reproductive Endocrinology of *Sycionia injentis* : Steroid ,Peptide and Terpenoid Hormones. *NOAA Tech.Rep.*, NMFS **106**:1-6.

*Chang,E.S.,Prestwich,G.D. and Bruce,M.(1990) Aminoacid sequence of a peptide with both molt and hyperglycemic activity in the lobster,*Homarus americanus*. *Biochem. Biophys.Res.Comm.*,**171**:818- 826.

Chan,H.H. and Lim,S.H.(1988) Effects of Progesterone on Ovarian Maturation of the white prawn, *Penaeus merguiensis* de Man. *Aquacult.Intern.Congr. Exploration*. Van Cour, Canada. p.63.

*Charniaux-Cotton,H.(1985) Vitellogenesis and its control in malcostracan Crustacea. *American Zool.*,**25**:197-206.

*Charniaux-Cotton,H.and Payen,G.(1988) Crustacean reproduction. In:H.Laufer and R.G.K. Downer (eds.),*Endocrin.Selected Invert. types*. Alan,R.Liss, New York. pp.279-303.

*Chaudenneret,P.J.(1956) Le systeme nervaux dela region gnathale de l'ecrensse *Cambarus affinis* (Say).*Ann.Sci.Nat.Zool.Biol. Anim.*,18:33-61.

*Chen,C.A.(1979) Preliminary report on the gonadal development and induced breeding of *Penaeus monodon* Fabricius. *MS Thesis*, Natn. Taiwan Univ.,Tapei. 43 pp.

*Chen,H.P.(1977) Report on maturation of *Penaeus monodon* Fabricius by eyestalk ablation and subsequent spawning and production of juveniles. *Chinese Fish.Mon.*, 294:3.

Chen,F.,Reid,B. and Arnold C.R.(1991) Maturing,Spawning and Egg Collecting of white Shrimp *Penaeus vannamei* Boon in a Recirculating System. *J.World Aquacult. Soc.*, 22:167-172.

*Cheung,T.S.(1969) The environment and hormonal control of growth and reproduction in adult female hone crab,*Menippe mercenaria*. *Biol.Bull.*,136:327-346.

Choy,S.C.(1987) Growth and Reproduction of Eyestalk Ablated *Penaeus canaliculatus* (Olivier,1811)(Crustacea:Penaeidae). *J.Exp.Mar.Biol.Ecol.*,122:93-107.

CMFRI (1994) Inducing Maturation of Penaeid Prawns. *CMFRI Res. Highlights 1993- 94*. pp.12-13.

Conte,F.S.,Duronslet,M.J.,Clark.W.H.and Parker,J.C.(1977) Maturation of *Penaeus stylirostris* (Stempson) and *Penaeus setiferus* (Linn.) in hypersaline water in Corpus Christi, Texas. *Proc.World Maricult.Soc.*,8:327-334.

*Couch,E.F. and Hagino,N.(1983) Correlation of progesterone and estradiol production by the mandibular organ and other tissues with egg development in the American lobster. *J. Cell Biol.*,97:158A (Abstract only).

Couch,E.F.,Hagino,N.and Lee,T.W.(1987) Changes in Estradiol and Progesterone Immunoreactivity in Tissues of the Lobster, *Homarus americanus* with Developing and Immature Ovaries. *Comp. Biochem.Physiol.*,87(A):765-770.

Cripe,G.M. (1994) Induction of maturation and spawning of pink shrimp,*Penaeus duorarum* by changing water temperature and survival and growth of young. *Aquaculture*, **128**:255-260.

Crocos,P.J. and Kerr,J.D.(1983) Maturation and spawning of the banana prawn, *Penaeus merguiensis* de Man (Crustacea: Penaeidae) in the Gulf of Carpentaria, Australia. *J.Exp.Mar.Biol. Ecol.*,**69**:37-59.

Crocos,P.J.and Kerr,J.D.(1986) Factors Affecting Induction of Maturation and Spawning of the tiger prawn, *Penaeus esculentus* (Hoswell), under Laboratory Conditions. *Aquaculture*, **58**: 203-214.

Cummings,W.C.(1961) Maturation and Spawning of the Pink Shrimp, *Penaeus duorarum* Burkenroad. *Trans.Am.Fish.Soc.*,**90**:462-468.

*Dakin,W.F.(1946) Life-history of a species of *Metapenaeus* in Australian coastal lake. *Nature*, **158**:99.

Dall,W., Hill,B.J., Rothlisberg,P.C. and Staples,D.J.(1990) *The Biology of Penaeidae*. In: J.H.Blaxter and A.J.Southward (eds.), *Adv.Mar.Biol.*. Acad. Press,New York. **XVII**: 251-275.

*D' Croz,L.,Wong,V.L.,Justin,G. and Gupta,M.(1988) Prostaglandins and Related Compounds from the Polychaete worm *Americomphis reesei* Fauchald (Onuphidae) as Possible inducers of Gonad Maturation in Penaeid Shrimps. *Rev. Biol. Trop.*,**36**:331-332.

*De Bruin,G.H.P.(1965) Penaeid prawns of Ceylon (Crustacea, Decapoda,Penaeidae). *Zool.Medele lingeu,Leiden,***41**:73-104.

*De'meusy,N.(1963) Rapportes entre mue et vitellogene'se chez le crabe, *Carcinus maenas* L. *Proc.XVI Int.Congr.Zool.*,**2**:118.

*De'meusy,N. and Veillet,A.(1952) De'clenchment de la punte chez le crabe, *Carcinus maenas* Pennant par l'ablation des pe'doncules oculaires. *C.R.Hebd Se'anc. Acad. Sci., Paris*, **234**:1224- 1226.

Derelle,E.,Groschude,J.,Meusy,J.J.,Junera,H.and Marian,M.(1986) ELISA titration of vitellogenin and vitellogen in the freshwater prawn *Macrobrachium rosenbergii* with monoclonal activity. *Comp.Biochem.Physiol.*,**85(B)**:1-4.

De Saint-Brisson,S.C.(1985) The mating behaviour of *Penaeus paulensis* Perez-Farfante, 1967 (Decapoda,Penaeidae). *Crustaceana*,**50**:108-110.

Devi,L.S.(1986) Growth and population dynamics of the Indian white prawn, *Penaeus indicus* H.M.Edwards from Kakinada.*Indian Acad.Sci.(Anim.Sci.)*,**95**:629-639.

Devi,L.S.(1987) Biology of the Indian White Prawn,*Penaeus indicus* H.M.Edwards from Kakinada,East Coast of India. *Indian J.Mar.Sci.*,**16**:246-248.

*Donahue,J.K.(1940) Occurrence of estrogens in the ovaries of certain marine invertebrates. *Endocrinology*,**27**:149-152.

*Donahue,J.K.(1948) Identification and biological determination of estrogens in the eggs of American lobster (*Homarus americanus*).*Proc.Soc.Exp.Biol.Med.*,**69**:179-181.

*Donahue,J.K.(1952) Studies on ecdysone in the American lobster (*Homarus americanus*). 1.The lobster eggs as a source of estrogenic hormone. *State of Maine Dept.Sea and Shore Fish.Res. Bull.*,No.8.

*Donahue,J.K.(1957) Chromatographic identification of lobster estrogens. *State of Maine Dept.of Sea and Shore Fish.Res. Bull.*,No.28.

*Drach,P.(1955) Syste'me' endocrine's pe'donculaire du ree'd'intermue et vitelloge'n'ese chez *Leander serratus* (Pennant). Crustace'Decapode. *C.R.Se'anc.Soc.Biol.*,**149**: 2079-2083.

*Downer,R.G.H. and Laufer,H.(1983) Endocrinology of insects. *Invertebrate endocrinology*. Alan,R.Liss,New York. I:707pp.

*Dunham,P.T.(1978) Sex pheromones in Crustacea. *Biol.Rev.*,**53**: 555-583.

Duronslet, M.J., Yudin,A.I., Wheeler and Clark,W.H.,Jr.(1975) Light and fine Structural Studies of Natural and Artificially Induced Egg Growth of Penaeid Shrimp. *Proc. World Maricult. Soc.*,**6**:105-111.

Dy-Penaflorida,V. and Millamena,O.M.(1990) Variation in the Biochemical Composition of *Penaeus monodon* Tissues during the Reproductive Cycle. *Bamidegh*,**42**:84-90.

Eastman-Reks,S.and Fingerman,M.(1984) Effects of Neuroendocrine Tissue and Cyclic AMP on Ovarian Growth *in vivo* and *in vitro* in the Fiddler Crab, *Uca pugilator*. *Comp.Biochem.Physiol.*, **79(A)**:679-684.

*Echalier,G.(1954) Recherches expe'rime'ntia sur le ro'le de l'organe Y dans la mue de *Carcinus maenas* (L.),Crustace' De'capode. *C.R.Hebd.Acad.Sci.,Paris,***238**:523.

Emmerson,W.D.(1980) Induced Maturation of *Penaeus indicus*. *Mar. Ecol. Progr. Ser.*, **2**: 121-131.

Emmerson,W.D.(1983) Maturation and Growth of Ablated and Unablated *Penaeus monodon* Fabricius. *Aquaculture*,**32**:235-241.

*Emmerson,W.D.,Hayes,D.P. and Ngonyami,M.(1983) Growth and maturation of *Penaeus indicus* under blue and green light. *South African J.Zool.*,**18**:71-75.

Fairs,N.J., Evershed,R.P., Quinlan,P.T. and Goad,L.J. (1989) Detection of Unconjugated and Conjugated Steroids in the Ovary, Eggs and Hemolymph of the Decapod Crustacean, *Nephrops norvegicus*. *Gen.Comp.Endocrinol.*,**74**:199-208.

Fairs,N.J.,Quinlan,P.T. and Goad,L.J.(1990) Changes in Ovarian Conjugated and Unconjugated Steroid Titers during Vitellogenesis in *Penaeus monodon*. *Aquaculture*, **89**:83-99.

Fakhfakh,M. and Cahu,C. (1990) Effect of dietary vitamin E on reproduction of penaeid shrimp: II.Analysis data. *World Aquacult.Soc.21 st ann.conf.*, p.8 (Abstract only).

*Fakhfakh,M,Villette,M.and Cahu,C.(1991) Effects of a-Tocopherol on Embryonic Development of *Penaeus indicus*: Antioxidant or Specific Role?. *ICES.CM. 1991/F: 35 ICES Counc.Meet.Pap.,***No.11**.

*Ferzou,J.P, Barbier,M. and Berreur-Bonnenfant,J.(1978) Biosynthese de la farnesylacetone- (E,-E) par les glandes androgenes du crabe *Carcinus maenas*. *Helv.Chim.Acta.,***61**:669-674.

*Fingerman,M.(1985) The Physiology and pharmacology of crustacean chromatophores. *American Zool.*, **25**:233-252.

Fingerman,M.(1987) The Endocrine Mechanisms of Crustacea. *J. Crust.Biol.*,**7**:1-24.

*Forster,G.R.(1951) The biology of the common prawn *Leander serratus* Pennant. *J.Mar. Biol.Assoc.UK.*,**30**:333-360.

*Galgani,M.-L.,Cuzon,G.,Galgani,F. and Goguenheim,J.(1989) Influence of the Diet on the Reproduction of *Penaeus indicus* in Captivity. *Aquaculture*,**81**:337-350.

Galois,R.G.(1984) Variations in Tissue Lipid Composition during Vitellogenesis in the Prawn *Penaeus indicus* Milne Edwards. *J.Exp.Mar.Biol.Ecol.*,**84**:155-166.

*Garcia,S. and Le Reste,L. (1981) Life cycles, dynamic exploitation and management of coastal penaeid shrimp stock. *FAO Fish. Tech.Pap.*,**203**:1-215.

*Gehring,W.B.(1974) Maturational changes in the ovarian lipid spectrum of the pink shrimp, *Penaeus duorarum* Burkenroad. *Comp.Biochem.Physiol.*,**49(A)**:511-524.

*George, B. and Khoo, H.W. (1989) Effects of estrogen and progesterone on vitellogenin levels into haemolymph of the shrimp, *Macrobrachium lanchesterii*. In:*Abstr.IV th Intern.Congr.Inv.Reprod.*, Nagoga, Japan. Intern.Soc.Inv.Reprod.,Maiz,Germany. p.57.

George,K.V.(1974) Some Aspects of Prawn Culture in the Seasonal and Perennial fields of Vypeen Island. *Indian J.Fish.*,**21**:1-19.

George,M.J.(1962) On the breeding of penaeids and the recruitment of their post larvae into the backwaters of Cochin. *Indian J.Fish.*,**94**:110-116.

George,M.J. and Rao,P.V. (1967) Distribution of sex ratios of penaeid prawns in trawl fishing off Cochin. *Proc.Symp.Crust.*. Mar.Biol.Assoc.India,Cochin,**III**:698-670.

George,M.J. and Rao,P.V.(1968) Observations on the development of the external genitalia in some Indian penaeid prawns. *J. Mar.Biol.Assoc.India*,**10**:52-70.

George,M.J.,Raman,K. and Nair,P.K.(1967) Observations on the offshore prawn fishery of Cochin. *Indian J.Fish.*,**10**:24-25.

*Gomez,R.(1965) Acceleration of gonads by implantation of brain in the crab *Paratelphusa hydrodromous*. *Naturwissenschaftu*, **9**:216.

Gopalakrishnan,P.,Raju,V.K,Pillai,K.M. and Thakar,S.J.(1987) On the Occurrence of Mature Penaeid Prawn *Penaeus merguiensis* in a Shallow Solar Salt Works Reservoir Along the Okhamanda Coast of Gulf of Kutch and Its Spawning in Laboratory. *Fish. Tech.*,**24**:12-13.

Grant,A.and Tyler,P.A.(1983) The Analysis of Data in Studies of Invertebrate Reproduction. I. Introduction and Statistical Analysis of Gonad Indices and Maturity Indices. *Int.J.Inv. Reprod.*,**6**:259-270.

*Gyary,J.C.,Kayama,M. and Murakami,Y.(1974) Lipid class distribution and fatty acid composition of prawn, *Penaeus japonicus*. *Bull.Japanese Soc.Sci.Fish.*,**40**: 1027-1032.

*Gyary,M,Kanazawa,A,Takaka,N.and Ceccaldi,H.J.(1976) Nutritional requirements of prawn. VI. Requirements for ascorbic acid. *Mem. Fac.Fish.Kagoshima Univ.*, **25**: 53-57.

Halder,D.D.(1978) Induced Maturation and Breeding of *Penaeus monodon* Fabricius under Brackishwater Pond Conditions by Eyestalk Ablation. *Aquaculture*,**15**: 171-174.

Halder,D.D.(1980) Breeding of *Penaeus monodon* Fabricius in Farm Pond under Captivity. *Proc.Natl.Symp.Shrimp Farming.* MPEDA, Cochin. pp.43-46.

*Hall,D.N.F.(1962) Observations on the taxonomy and biology of some Indo-West Pacific penaeidae (Crustacea,Decapoda). *Colonial Office Fish.Publ.*, Her Majesty Stationary Office,London. **17**:1-229.

*Hampshire,F. and Horn,D.H.S.(1966) Structure of crustecdysone, a crustacean molting hormone. *Chem.Comm.*,**2**:37-38.

Hansford,S.W.,Mcguren,J.J. and Marsden,G.E.(1993) The Effect of Substrate Type on the Ovarian Maturation of *Penaeus japonicus* Bate. *Asian Fish.Sci.*,**6**:283-293.

*Harrison,K.E.(1990) The role of nutrition in maturation, reproduction and empryogenic development of decapod crustaceans : A review. *J.Shellfish Res.*,**9**:1-28.

*Hazel,C.M.(1986) Steroidogenesis in the female crab, *Carcinus maenas*. *Ph.D.Thesis.* Univ.Liverpool. pp81-121.

*Hazlett,B.A.(1975) Ethological analysis of reproductive behaviour in marine crustacean. *Pubbl.Staz.Zool.,Napoli*,**39**:677-695.

He,H. and Lawrence,A.L.(1993) Vitamin E Requirement of *Penaeus vannamei*. *Aquaculture*, **118**:245-255.

*Heldt,J.H.(1938) La reproduction chez les crustsce's de'capodes de la femelle des pe'neide's. *Annal.Inst.Oceanogr.,Paris*, **18**: 31-206.

Hillier,A.G.(1984) Artificial Conditions Influencing the Maturation and Spawning of Subadult *Penaeus monodon* (Fabricius). *Aquaculture*,**36**:179-184.

*Hinsch,G.W.(1977) Fine structural changes in the mandibular organ gland of the male spider crab, *Libinia emarginata* (L.) following eyestalk ablation. *J.Morph.*,**154**: 307-315.

*Hinsch,G.W.(1981) Effects of juvenile hormone mimics on the ovary in the immature spider crab,*Libinia emarginata.Intern. J.Inv.Reprod.*,**3**:237-244.

*Hinsch,G.W. and Bennet,D.C.(1979) Vitellogenesis stimulated by thoracic ganglion implanted into destalked immature spider crab, *Libinia emarginata. Tiss.Cell*, **11**: 345-351.

Hirasawa,Y.(1985) Economics of shrimp culture in Asia. **In**: Y.Taki, J.H.Primavera and J.A.Llobrera (eds.),*Proc.Ist Intern.Conf.Cult.Penaeid Prawn/Shrimps*, SEAFDEC, Iloilo City, Philippines. pp.131-150.

*Homola,E.,Landau,M.and Laufer,H.(1989) An *in vitro*assay for the regulation of methyl farnysoate synthesis in desegregated mandibular organ cells from L*ibinia emarginata* and the effect of serotin *.Biol.Bull.*,**176**:69.

*Hudinaga,M.(1942) Reproduction,Development and rearing of *Penaeus japonicas* Bate. *Japanese J.Zool.*,**10**:305-393.

*Idyll,C.P.(1971) Induced maturation of ovary and ova in pink shrimp.*Comm.Fish.Rev.*, **33**:20-27.

Jayachandran,K.V. and Jose,T.M.(1993) Effects of unilateral eyestalk ablation and male deprivation on ovarian maturation and ovulation in *Macrobrachium idella idella* (Hilgendorf,1898) during the post breeding season. *Indian J.Comp.Anim. Physiol.*, **11**:35-38.

Jeng,S.S.,Wan,M.W.C.M.,and Chang,C.F.(1987) Existence of an Estrogen like Compound in the Ovary of the Shrimp *Parapenaeus fissurus. Gen.Comp. Endocrinol.*,**36**:211-214.

*Jerlov,N.G. (1970) Light: general introduction. In: O.Kinne (ed.), *Marine Ecology*, Wiley Interscience,London. pp.95-102.

*Johnson,M.,Carry,F.and McMillan,R.M.(1983) Alternative path- ways of arachidonate and leukotrienes. In: P.N.Campell and R.D. Marshall (eds.),*Essays in Biochem.*. Acad.Press,NY.**19**: 40- 141.

*Johnson,M.C. and Fielding,J.C. (1956) Propagation of the white shrimp (*Penaeus setiferus*) in captivity. *Tulane Stud.Zool.*, **4**:175-190.

*Joshi,P.K.(1980) Reproductive physiology and neurosecretion in some Indian marine prawns. *Ph.D.Thesis*. Marathwada Univ., Aurangabad.

*Juchault,P. and Legrand,J.J. (1967) Contribution a'le'etude du controle neurohumarale de la glande androge'ne chez les onoschides. *C.R.Hebd.Se'anc.Acad.Sci.*, *Paris*, **264**:1644-1646.

*Junera,H.,Zerbib,C.,Marian,M.and Meusy,J.-J.(1977) Evidence for control of vitellogenin synthesis by an ovarian hormone in *Orchestia gammarella* (Pallas), Crustacea Amphipoda. *Gen.Comp. Endocrinol.*,**31**:457-462.

Kallen,J.and Meusy,J.-J.(1989) Do the Hormones VIH (Vitellogenin Inhibiting Hormone) and CHH(Crustacean Hyperglycemic Hormone) of Crustaceans have a Common Precursor ? Immunolocalization of VIH and CHH in the X-Organ Sinus Gland Complex of the Lobster,*Homarus americanus.J.Inv.Reprod.Dev.*,**16**: 43-52.

*Kamaguchi,Y.(1971) Studies of moulting in freshwater prawn *Macrobrachium paucidens*. II. Effect of eyestalk removal in relation to the rate of ovarian growth. *J.Fac.Sci.Hokkaido Univ. Ser.Zool.*,**18**:24-31.

*Kanazawa,A.(1982) Control of ovarian maturation and spawning of aquatic animals. *Suisan aku Ser.*.Tokyo.**39**:80-89.

Kanazawa,A.(1985) Nutrition of penaeid prawns and shrimps. In: Y.Taki,J.H.Primavera and J.A.Llobrera (eds.), *Proc.I st Intern.Conf.Cult.Penaeid prawns/shrimps*. SEAFDEC, Iloilo City. pp.123-130.

Kanazawa,A.(1990) Effect of clam diet on ovary maturation of *P.japonicus. World Aquacult. Soc.21 st Ann. Conf.*. p.7 (Abstract only).

*Kanazawa,A. and Teshima,S. (1971) *In vitro* conversion of cholesterol to steroid hormones in the spiny lobster, *Panulirus japonicus. Bull.Jap.Soc.Sci.Fish.,* **37**: 891-898.

Kanazawa,A., Chim,L. and Laubi,A.(1988) Tissue uptake of radioactive cholesterol in the prawn *Penaeus japonicus*, Bate during induced ovarian maturation. *Aquat.Living Resour.,***1**:85-91.

*Kanazawa,A,Tanak,N,Teshima,S.and Kashiwada,K.(1971a) Nutritional requirements of prawn.II.Requirements for sterols. *Bull. Japanese Soc.Sci.Fish.,***37**:211-215.

*Kanazawa,A,Tanak,N,Teshima,S.and Kashiwada,K.(1971b) Nutritional requirements of prawn.III.Utilization of sterols. *Bull. Japanese Soc.Sci.Fish.,***37**:1015-1019.

*Karlson,P.(1956) Biochemical studies on insect hormones,vitamins. *Hormones,* **14**: 227-266.

Kathirvel,M.(1985) Occurrence of Maturing females of Kuruma Prawn, *Penaeus japonicus* in the Mariculture Farm at the Muthukkadu. *Mar.Fish.Inf.Ser.T&S,* **65**:12-17.

Kathirvel,M.and Selvaraj,V.(1987) On experimental field collection and field culture of king prawn *Penaeus latisulcatus. Indian J.Fish.,***34**:365-373.

Kathirvel,M. and Selvaraj,V. (1989) Attainment of Maturity in Pond Reared Kuruma Prawn *Penaeus japonicus* Bate. *J.Mar.Biol. Assoc.India,***31**:110-116.

Keeley,-L.L.(1991) Speculations on Regulation of Shrimp Reproduction Through Peptide Biotechnology. *Progr.and Abstr.Sec. Mar.Biotech.Conf.-IMBC-'91.* p.65.

Kelemec,J.A. and Smith,I.R.(1980) Induced Ovarian Development and Spawning of *Penaeus plebejus* in a Recirculating Laboratory Tank after Unilateral Enucleation. *Aquaculture,* **21**:56-62.

Kelemec,J.A. and Smith,I.R.(1984) Effects of low Temperature Storage and Eyestalk Enucleation of Gravid Eastern King Prawns,*Penaeus plebejus,*on Spawning,Egg Fertilization and Hatching. *Aquaculture,***40**:67-76.

*King,J.E.(1948) A study of the reproductive organs of the common marine shrimp *Penaeus setiferus* (Linnaeus). *Biol.Bull.,***94**: 244-262.

*Kinsella,J.K.(1988) Food lipid and fatty acids: importance in food quality,nutrition and health. *Food Tech.*,**42**:124-145.

Klek-Kawinska,E. and Bomirski,A.(1975) Ovary inhibiting in the shrimp (Crangon crangon) eyestalks during the reproductive cycle. *Gen.Comp.Endocrinol.*,**25**:9-13.

Knowles,F.G.W.andCarlisle,D.B.(1956) Endocrine control in crustacea. *Biol.Rev.*, **31**: 396-473.

Koskela, R.W., Greenwood, J.G. and Rothlisberg, P.C. (1992) The Influence of Prostaglandin E 2 and the Steroid Hormones,17- a Hydroxy Progesterone and 17-b Estradiol on Moulting and Ovarian Development in the Tiger Prawn, *Penaeus esculentus* Haswell, 1879 (Crustacea:Decapoda). *Comp.Biochem.Physiol.*, **101(A)**: 295-299.

Krishnamurthy,V. and Ganapathy,R.(1985) On the Occurrence of Maturing *Penaeus indicus* in Brackishwater Environment. *Seafood Export J.*,**17**:13-14.

*Kulkarni, G.K. and Fingerman,M. (1992) Effects of 5-Hydroxy tryptamine Agonists on Ovarian Development in the Fiddler Crab *Uca pugilator*. *Comp.Biochem. Physiol.*, **101(C)**:419-423.

Kulkarni, G.K. and Nagabhushanam, R. (1980) Role of Ovary Inhitibing Hormones from Eyestalks of Marine Penaeid Prawns (*Parapenaeopsis hardwickii*) during Ovarian Development Cycle. *Aquaculture*,**19**:13-19.

Kulkarni,G.K.,Glade,L. and Fingerman,M.(1991) Oogenesis and effect of neuroendocrine tissues in *in vitro* synthesis of protein by the ovary of the red swamp crayfish *Procambarus clarkii*. (Gaillard). *J.Crust.Biol.*,**11**:513-522.

Kulkarni,G.K.,Nagabhushanam,R. and Joshi,P.K.(1979) Effect of progesterone ovarian maturation of the penaeid prawn, *Parapenaeopsis hardwickii*. *Indian J.Exp.Biol.*, **17**:986-987.

Kulkarni,G.K.,Nagabhushanam,R., Amaldoss,G., Jaiswal,R.G. and Fingerman,M. (1992) *In vivo* Stimulation of Ovarian Development in the Red Swamp Crayfish, *Procambarus clarkii* (Gaillard), by 5-Hydroxytryptamine. *Inv.Reprod.Dev.*,**21**: 231-240.

Kumari,S.S.and Pandian,T.J.(1987) Effects of Unilateral Eyestalk Ablation on Moulting,Growth,Reproduction and Energy Budget of *Macrobrachium nobilii*. *Asian Fish.Sci.*,**1**:1-17.

Kurup,K.N.P. and Adiyodi,R.G. (1980) Pattern of regeneration during the breeding in the Crab,*Paratelphusa hydrodromous* (Herbst).In:T.Subramoniam and S. Varadarajan (eds.), *Progr.Inv.Reprod.Aquacult.*,New Century Printers,Madras. pp.:25-26.

Kurup,N.S. and Rao.P.V.(1974) Population characteristics of the important marine prawns of Ambalappuzha, Kerala. *Indian J. Fish.*,**21**:183-210.

*Lachaise,F.and Hoffman,J.A.(1977) Ecdysone et development ovarienne chez un De'capode, *Carcinus maenas. C.R.Acad.Sci.,Paris*, **285**:701-704.

*Lachaise,F.,Gouderman,M.,Hetru,C.,Kappler,C. and Hoffman,J.A. (1981) Ecdysteroid and ovarian development in the shore crab, *Carcinus maenas. Hoppe Scylers Zeitschrift Physiol.Chem.*,**362**: 521-529.

Landau,M,Laufer,H.and Homola,E.(1989) Control of methylfarnesoate synthesis in mandibular organ of the crayfish *Procambarus clarkii*: evidence for peptide neurohormone with dual function. *Inv.Reprod.Dev.*,**16**:165-168.

*Laubier-Bonichon,A.(1975) Ecophysiogie induction de la maturation sexuelle et ponte chez la crevette *Penaeus japonicus* Bate en miliue controle. *C.R.Acad.Sci.,Paris*, **281**: 2013-2016.

*Laubier-Bonichon,A.(1978) Ecophysiology of the prawn *Penaeus japonicus* three years experiments in control conditions. *Oceanol.Acta.*,**1**:135-150.

*Laubier,A. and Laubier,L.(1976) Reproduction control with the shrimp *Penaeus japonicus. FAO Tech.Conf.Aquacult.*,Kyoto.p.6.

*Laubier-Bonichon,A. and Laubier,L.(1979) Reproduction controle chez la crevette *P.japonicus*. In: T.V.R.Pillay and W. Dill (eds.), *Adv.Aquacult.*, Fishing News Books Ltd. Surrey.pp.273- 277.

Laufer,H.and Landau,M.(1991) Endocrine Control of Reproduction in Shrimp and other Crustacea. In:P.F.DeLoach,W.J.Dougherty and M.A.Davidson (eds.), *Frontiers of Shrimp Research*. Elsevier Science Publishers, Amsterdam. pp.65-82.

*Laufer,H.,Borst,D.W.,and Homola,E.(1986) The synthesis and regulation of methyl farnesoate, a new juvenile hormone for crustacean reproduction. In: M.Porchet, J.C. Andrien and A. Dhainaut (eds.),*Adv.Inv.Reprod.*, Elsevier Science Publishers, Amsterdam. pp.135- 143.

Laufer,H.,Homola,E. and Landau,M.(1992) Hormonal Regulation of Reproduction in female Crustacea.*NOAA Tech.Rep.*NMFS,**106**:89-97.

*Laufer,H.,Homola,E.and Landau,M.(1987a) Control of methyl Farnesoate synthesis in crustacean mandibular organs. *American Zool.*,**27**:69A (Abstract only).

*Laufer,H., Borst,D., Baker,F.C., Carrasco,C.,Sinkins,M., Renter,C.C., Tsai, L.W. and Schooley,D.A. (1987b) Identification of a Juvenile Hormone like Compound in a Crustacea. *Science*,**235**: 202-205.

*Laufer,H.,Borst,D.W.,Carrasco,C.,Barber,F.C.and Schooley,D.A. (1984) The detection of juvenile hormone in crustacea. *American Zool.*,**24**:34A (Abstract only).

Lawrence,A.L.,Ward,D.,Missler,S.,Brown, A., McVey, J.P. and Middleditch,B.S.(1979) Organ indices and biochemical level of ova from penaeid shrimp maintained in captivity versus those captured from the wild. *Proc.WorldMaricult.Soc.*,**10**: 453-463.

Lawrence,A.L., Akamine,Y., Middleditch,B.S., Chamberlain,G. and Hatchins,D.(1980) Maturation and Reproduction of *Penaeus setiferus* in Captivity. *Proc.World Maricult. Soc.*, **11**: 481-487.

Lee,P.F. and Puppione,D.L.(1988) Lipoprotein I and II from the haemolymph of the blue crab,*Callinectes sapidus*:lipoprotein II assessment with vitellogenesis. *J.Exp.Zool.*, **248**:278-289.

*Le Roux,A.(1968) Description d'organes mandibulaires on veaux chez les Crustace's Decapodes. *C.R.Hebd.Acad.Sci.,Paris*,**266D**: 1414-1417.

*Le Roux,A.(1983) Revition de l'organe mandibulaires' l'ablation des pe'doncules occular chez les larves et les juveniles de *Paleamonetes varians* (Leach)(Decapoda Natantia). *C.R.Acad. Sci.,Paris*,**296**:697-700.

*Liang,V,Zhang,N.,Cao,D.,Gao,H.,Liu,R.and Zhang,W.(1983) Studies on the induction of ovarian maturity and spawning of *Penaeus orientalis* Kishinouye by eyestalk ablation. *Oceonol.Limnol. Sin.*,**14**:38-147.

Lichatowich,T.,Smalley,T.and Mate,F.D.(1978) The Natural Reproduction of *Penaeus merguiensis* (De Man,1888) in the Earthern Pond in Fiji. *Aquaculture*,15:377-378.

Lin,-M and Ting,-Y.(1986) Observations of Poor Hatching in the Unilateral Eyestalk Ablated females of *Penaeus monodon*. *Bull. Jap.Soc.Sci.Fish.*,52:355.

*Lisk,R.D.(1961) Estradiol-17b in the eggs of American lobster, *Homarus americanus*. *Can.J.Biochem.Physiol.*,39: 659-662.

*Liu,R (1983) Shrimp mariculture studies in China. In: G.L. Rogers, R.Day and A.Lim (eds.), *Proc. 1 st Intern.Conf.Warmw. Aquacult.-Crust.*. Brighen Young Univ., Hawaii. pp.82-90.

*Lockwood,A.P.M.(1968) *Aspects of the physiology of Crustacea*. Olivier and Boyd, London.

*Lowe,M.E.(1961) The female reproductive cycle of crayfish *Cambarellus shufeldti*: The influence of environmental factors. *Tulane Stud.Zool.*,8:157-176.

Luis,O.J. and Ponte,H.C.(1993) Control of Reproduction of the Shrimp *Penaeus kerathurus* held in Captivity. *J.World Aquacult.Soc.*,24:31-39.

Lumare,F.(1979) Reproduction of *Penaeus kerathurus* using Eyestalk Ablation. *Aquaculture*, 18:203-214.

Lumare,F. (1981) Artificial reproduction of *Penaeus japonicus* Bate as a basis for the mass production of eggs and larvae. *J.World Maricult.Soc.*,12:338-344.

Lumare,F.(1990) Crucial Points into and Commercial Production of Shrimps. In: R.Idos, L.Tort and P.Torres(eds.), *Mediterranean Aquaculture*. Ellis Harwood Books. London. pp.21-43.

Lytle,J.S. and Lytle,T.F.(1990) Fattyacid Composition and Variations in Individual Bloodworms. *J.World Aquacult.Soc.*,21: 314-318.

Lytle,J.S,Lytle,F.and Ogle,J.T.(1990) Polyunsaturated Fatty acids as a Comparative Tool for in Assessing Maturation Diets of *Penaeus vannamei. Aquaculture*,89:287-299.

*Magarelli,Jr.,P.C.(1981) Nutritional and behavioural component of reproduction in the blue shrimp,*Penaeus stylirostris*, reared under controlled environment condition. *Ph.D. Dissertation*, Univ.Arizona,Arizona.81pp.

Makinouchi,S. and Primavera,J.H. (1987) Maturation and Spawning of *Penaeus indicus* using Different Ablation Methods. *Aquaculture*,**62**:73-81.

Manisseri,M.K.and Manimaran,C.(1981) On the fishery of the Indian white prawn *Penaeus indicus* H.Milne Edwards along the Tinnevelly coast, Tamilnadu. *Indian J. Fish.*, **28**:208-216.

*Marchiori,M.F. and Boff,M.H.(1983) Induced maturation, spawning and larva culture of the pink shrimp *Penaeus paulensis* Perez- Farfante.*Mem.Assoc.Latin-American Acuicult.*,**5**:331-337.

Marsden,G., McGuren,J.M.,Sarac,H.Z., Neill,A.R.,Brock,I.J. and Palmer,C.L.(1992) Nutritional Composition of some Natural Marine Feeds used in Prawn Maturation. In:G.L.Allan and W.Dall (eds.), *Proc.Aquacult.Nutri.Workshop*. pp.82-85.

Marte,C.L.(1982) Seasonal variation in food and feeding of *Penaeus monodon* Fabricius (Decapoda,Natantia). *Crustaceana*,**42**: 250-255.

*Mattson,M.P. and Spaziani,E.(1985) 5-tryptamine mediate release of the moult inhibiting hormone activity from isolated crab eyestalk ganglia. *Biol.Bull.*,**169**: 246-255.

Menasveta,P., Piyatiratitivorakul,S.,Rungsupa,S., Moree,N. and Fast,A.W.(1993) Gonadal Maturation and Reproductive Performance of Giant Tiger Prawn (*Penaeus monodon*) from the Andaman Sea and Pond-reared Sources in Thailand. *Aquaculture*,**116**:191- 198.

Menasveta,P.,Sangpradup,S.,Piyatiratitivorakul,S. and Fast,A.W. (1994) Effects of broodstock size and source on ovarian maturation and spawning of *Penaeus monodon* Fabricius from the Gulf of Thailand. *J.World Aquacult.Soc.*, **25**:41-49.

*Mendoza-Alfaro,-R.E.(1992) Study of stimulation of penaeid shrimp vitellogenin and its stimulation by means of heterologous and homologous factors. *Ph.D. Thesis*, Bretgene Occidental Univ., Brest,France. 202pp.

Middleditch,B.S.,Missler,S.R.,Hinsch,H.B.,Chang,E.S.,McVey,J.- P.,Brown,A. and Lawrence,A.L.(1980) Maturation of penaeid shrimp: lipids in the marine food web. *Proc.World Maricult. Soc.*,**11**:463-470.

Middleditch,B.S., Missler,S.R., Ward,D.G., McVey,J.P., Brown,A. and Lawrence,A.L. (1979) Maturation of Penaeid Shrimp : Dietary Fattyacids. *Proc.World Maricult. Soc.*, **10**:472-476.

Millamena,S.M.(1989) Effect of Fattyacid Composition of Broodstock Diet on Tissue Fattyacid Patterns and Egg Fertilization and Hatching in Pond Reared *Penaeus monodon. Asian Fish.Sci.*, **2**:127-134.

Millamena,S.M. and Pascual,F.P.(1990) Tissue Lipid Content and Fattyacid Composition of *Penaeus monodon* Fabricius Broodstock from Wild. *J.World Aquacult. Soc.*, **21**:116-121.

Millamena,O.M.,Primavera,J.H.,Pudadera,R.A. and Cabarello,R.V. (1986) The effect of diet on the reproductive performance of pond-reared *Penaeus monodon* Fabricius broodstock. In:J. L.Mclean,L.B.Dizon and L.V.Hosillo (eds.),*The 1 st Asian Fish. Forum*,Asian Fish.Soc.,Manila. pp.593-596.

Millamena,O.M.,Pudadera,R. and Catacutan,M.R.(1985) Variations in Tissue lipid Content and Fattyacid Composition during Ovarian Maturation of Unablated and Ablated *Penaeus monodon* Broodstock.In:Y.Taki,J.H.Primavera and J.A.Llobrera (eds.), *Proc.1 st Int.Sem.Cult.Penaeid Prawns/Shrimps*. SEAFDEC,Iloilo City, Philippines. p.116 (Abstract only).

Mohamed,K.H. and Rao,P.V.(1971) Estuarine Phase in the Life History of the Commercial Prawns of the West Coast of India. *J.Mar.Biol.Ass.India*,**13**:149-161.

Mohamed,K.S. and Diwan,A.D.(1991) Neuroendocrine Regulation of Ovarian Maturation in the Indian White Prawn *Penaeus indicus* H.Milne Edwards. *Aquaculture*, **98**:381-393.

Mohamed,K.S. and Diwan,A.D.(1992) Biochemical Changes in Different Tissues during Yolk Synthesis in Marine Prawn *Penaeus indicus* H.Milne Edwards.*Indian J.Mar. Sci.*, **21**:30-34.

* Morris,M.C. and Bennett,I.(1952) The life history of a penaeid prawns (*Metapenaeus*) breeding in a coastal lake (Tuggerah, New South Wales). *Proc.Linnaen Soc. New South Wales*. **76**: 164- 182.

* Motoh,M.(1981) Studies on the fisheries biology of the giant prawn,*Penaeus monodon* in the Philippines.*SEAFDEC Tech.Rep.*,**7**: 164-182.

MPEDA (1994) *Handbook on shrimp farming.* MPEDA, Kochi. 98 pp.

Murugadass,S.,Mathavan,S.,Marian,M.P. and Pandian,T.J.(1988) Influence of Eyestalk Ablation on Growth and Egg Production in *Macrobrachium malcomsonii.* In:M.M.Joseph (ed.), *Proc. 1 st Indian Fish.Forum.* Asian Fish.Soc., Indian Branch, Mangalore. pp. 111-114.

Muthu,M.S.(1983) Penaeid Broodstock Development and Management. *Proc.Natl.Symp. Shri.Seed Prodn.Hatch.Manag..*MPEDA. Cochin. pp.97-116

Muthu,M.S. and Laxminarayana,A.(1977) Induced Maturation and Spawning of Indian Penaeid Prawns.*Indian.J.Fish.,*24:172-180.

Muthu,M.S. and Laxminarayana,A.(1979) Induced Breeding of the Indian White Prawn *Penaeus indicus. Mar.Fish.Inf.Ser.T&S. Ser.,*9:6.

Muthu,M.S. and Laxminarayana,A.(1982) Induced Maturation of Penaeid Prawns- A review. *Proc.Symp.Coast.Aquacult..* Mar. Biol.Assoc.India.,Cochin, 1:16-27.

Muthu,M.S. and Manickam,P.E.S.(1973) On the Occurrence of Mature Specimen of *Metapenaeus burkenroadi* Kubo in the Pulikat Lake. *Indian.J.Fish.,*20:214-215.

Muthu,M.S.,Laxminarayana,A. and Mohamed,K.H.(1984) pH as a Factor Influencing Maturation of *Penaeus indicus* in Captivity. *Indian.J.Fish.,*31:217-222.

Nagabhushanam,R. and Kulkarni,G.K.(1981) Effect of exogenous testosterone on the androgenic gland and testis of a marine penaeid prawn,*Parapenaeopsis hardwickii* (Miers)(Crustacea,Decapoda,Penaeidae). *Aquaculture,*23:19-27.

Nagabhushanam,R.and Joshi,P.K.(1986) Effect of the environmental factors on the ovarian development and spawning of the penaeid prawn,*Parapenaeopsis stylifera.*In:M.F.Thompson, R.Sarojini and R.Nagabhushanam (eds.), *Biol.Benthic Mar.Organ.: Techniques as applied to the Indian Ocean.* Oxford & IBH Publishing Co.Pvt.Ltd.,New Delhi. pp 55-60.

Nagabhushanam,R.,Joshi,P.K. and Kulkarni,G.K. (1980) Induced spawning of in prawn *Parapenaeopsis stylifera* (H.Milne Edwards) using a steroid hormone 17 Hydroxy progesterone. *Indian J.Mar.Sci.,*9:227.

Nagabhushanam,R.,Joshi,P.K. and Kulkarni,G.K.(1982) Induced spawning in *Parapenaeopsis stylifera* (H.Milne Edwards) using a steroid hormone,17-Hydroxy progesterone.*Proc.Symp. Coast. Aquacult.*,Mar.Biol.Assoc.India,Cochin.1:37-39.

Nagabhushanam,R., Joshi,P.K. and Sarojini,R.(1987) Effect of Exogenous steroids on reproduction in *Parapenaeopsis stylifera. Adv.Aquat.Biol.Fish.*. pp201-206.

Nagabhushanam,R.,Machale,P.R. and Sarojini,R.(1988) Presence of ovarian stimulating factors in brain and thoracic ganglion of freshwater prawn,*Macrobrachium kistensis. J.Zool.Res.*,1:17-21.

Nagabhushanam,R.,Machale,P.R. and Sarojini,R.(1989) Pheromonal and hormonal control of reproduction in the freshwater prawn, *Macrobrachium kistensis. Proc. Indian Acad.Sci.*,98: 95-98.

Nascimento,I.A.,Bray,W.A.,Leung-Trujillo,J.-R. and Lawrence,A. (1991) Reproduction of ablated and unablated *Penaeus schmitti* in captivity using diets consisting of fresh frozen natural and dried formulated feeds. *Aquaculture*,99: 387-398.

*Nelson,K.(1991) Scheduling of reproduction in relation to molting and growth in malacostrocan crustaceans. In: A. Webster and A. Kerry (eds.), *Crustacean egg Production* European Aquacult.Soc.,Bredene,Crust.Iss.,VII:77-166.

*NRC (1983) *Nutrient requirements of warmwater fish and shellfish.* National Acad. Press, Washington,D.C. Revd.ed. 102 pp.

*Nurjana,M.L. and Yang,W.T.(1976) Induced gonad maturation,spawning and post larval production of *Penaeus merguiensis* de Man. *Bull.Shri.Cult.Res.Cent.*,Jepara. II:177-186.

*Ogle,J.T.(1988) Survey of 18 *Penaeus vannamei* maturation laboratory managers. *Gulf Coast Res.Lab. Consortium US shri.Farm. Progr.maturation Workshop*,6-7 April, 1988.

Ogle,J.T.(1992a) A review of the current (1992) state of our knowledge concerning reproduction in open thelycum shrimp with emphasis on *Penaeus vannamei. Invert. Reprod.Dev.*,22: 267-274.

Ogle,J.T.(1992b) The effect of salinity on spawning frequency of *Penaeus setiferus* in aquaria. *Gulf Coast Res.Rep.*,8: 427- 429.

Ollevier,F.,De Clarcke,D.,Diederik,K.H. and De Loof,A. (1986) Identification of non-ecdysteroid in the haemolymph of both male and female *Astacus leptodactylus* (crustacea) by gas chromatographic-mass spectrometry. *Gen.Comp.Endocrinol.*,**61**: 214-228.

O'Sullivan,D.(1994) Domestication of prawn spawners to underpin selective breeding programme. *Austasia Aquacult.*,**8**(3): 11-14.

*Otsu,T.(1960) Precocious development of the ovaries in the crab,*Potamon dehaani*, following implantation of thoracic ganglion. *Annot.Zool.Japan*,**33**:90-96.

*Otsu,T.(1963) Bihormonal control of sexual cycle in the freshwater crab,*Potamon dehaani*. *Embryologica*,**8**:1-20.

Ottogalli,L.,Galinie,C. and Goxe,D.(1988) Reproduction in captivity of *Penaeus stylirostris* over ten generations in New Caledonia. *J.Aqua.Trop.* 3:111-125.

*Pandian,T.J.(1967) Changes in chemical composition and calorie content of developing eggs of *Crangon crangon*. *Helgolander Wiss.Meersutr.*,**16**:216-227.

Pandian,T.J. and Sindukumari,S.(1985) Does eyestalk ablation induce hyperplasia ?. *Curr. Sci.*,**54**:1281-1282.

*Panikkar,N.K. and Menon, M.K.(1956) Prawn fisheries of India. *Proc.Indo-Pacific Fish. Counc.*, **6**:328-344.

*Panouse,J.B.(1943) Influence de l'ablation de pe'doncule oculaire sur la croissance de l'ovarie chez la crevette *Leander serratus*. *C.R.Acad.Sci.,Paris*,**217**:553-55.

*Panouse,J.B.(1944) L'action de la glande du sinus sur l'ovarie chez la crevette *Leander*. *C.R.Acad.Sci.,Paris*,**218**:293-294.

*Panouse,J.B.(1946) Recherches sur les pheromones humoraux chez les crustace's l'adaptation chromatographique et la croissance-ovarienne chez la crevette *leander serratus*. *Ann.Inst. Oceanogr.Monaco*, pp.23-65.

*Passano,L.M. (1960) Molting and its control. **In**: W.H.Waterman (ed.),*The physiology of crustacea*. Acad.Press,New York. **I**: 473-536.

*Paulus,J.E.(1984) Hormonal control of vitellogenesis in a decapod crustacean (Brachyura).*Ph.D.Dissertation*,Univ.Connecticut.

Paulus,J.E. and Laufer,H.(1987) Vitellogenesis in the hepatopancreas of *Carcinus maenas* and *Libinia emarginata* (Decapoda,Brachyura). *Int.J.Inv.Reprod.Dev.*, 11: 29-44.

*Payen,G.G. and Costlow,J.D.(1977) Effect of a juvenile hormone mimic on male and female gametogenesis of the mud crab *Rhithropanopeus harrissi*. *Biol.Bull.*,152: 199-208.

*Payen,G.G,Costlow,J.D.and Charniaux-Cotton,H.(1967) Recherches sur le de la neuroendocrinesecretion dans la differentiation sexuelle du crabe,*Callinectes sapidus* Rathbun. *C.R.Acad. Sci.,Paris*,264:2148-2151.

*Payen,G.G.,Costlow,J.D. and Charniaux-Cotton,H.(1971) Etude comparative del' ultrastructure des glandes androge'ne's de crabe, normaux et pe'doculectimised pendant lavia lawaire on apre's la puberte' chez les espece's: *Rhithropanopeus harrissi* (Gould) et *Callinectes sapidus* Rathbun. *Gen.Comp.Endocrinol.*,17: 526-542.

*Perez-Farfante,I.(1969) Western Atlantic shrimps of the genus *Penaeus*. *Fish.Bull.*, 67:461-591.

*Persis,B. and Sarojini,R.(1985) Ovary inhibiting hormone activity of the eyestalks of the freshwater prawn *Caridina rajadhari*. *Uthar Pradhesh J.Zool.*,5:159-163.

Petriella,A.M. and Diaz,A.C.(1987) Influence of eyestalk ablation on moulting and gonadal maturation of the Argentine prawn *Artemesia longinaris* Bate. *J.Aqua. Trop.*, 2:17-24.

*Poernomo,A.and Hamami,E.(1983) Induced gonad maturation,spawning and hatching of eyestalk ablated pond-grown *Penaeus monodon* in a recirculating water environment. In:G.L. Rogers,R.Day and A.Lim (eds.), *Proc.Intern.Conf. Warmwater Aquacult.Crust.*, Brighen Young Uni., Hawaii. pp.412-419.

Ponnuchamy,S.,Reddy,R.S. and Sakuntala,K.(1981) Effect of eyestalk ablation on growth and food conversion efficiency of the freshwater prawn *Macrobrachium lachesterii* (De Man). *Hydrobiologica*,77:77-80.

*Posadas,R.A.(1986) The effect of salinity on the maturation and spawning of ablated *Penaeus monodon* (Fabricius). *MS Thesis*. Univ.Philippines,Visayas.50pp.

*Pradielle-Rouquette,M.(1975) Ro'le de la tempe'rature dans la function de reproduction des femelles du crabe *Pachygrapsus marmoratus* (Fabricius). *C.R.Acad.Sci., Paris*, **281**:711-713.

Primavera,J.H.(1978) Induced Maturation and Spawning in five month old *Penaeus monodon* Fabricius by eyestalk ablation. *Aquaculture*,**13**:355-359.

Primavera,J.H.(1979) Notes on courtship and mating behaviour in *Penaeus monodon* Fabricius (Decapoda: Natantia).*Crustaceana*, **37**:287-292.

*Primavera,J.H.(1980) Sugpo (*Penaeus monodon*) broodstock. *Aquacult.Guide*. Aquacult. Dept., SEAFDEC,Iloilo City. **1**:10-15.

Primavera,J.H.(1982) Studies on Broodstock of sugpo *Penaeus monodon* Fabricius and other penaeids at the SEAFDEC Aquaculture Department. *Proc.Symp.Coast. Aquacult.*. Mar.Biol.Assoc., India.**1**:28-36.

Primavera,J.H.(1985) A Review of Maturation and Reproduction in closed thelycum Penaeids. In:Y.Taki,J.H.Primavera and J.A.Llobrera (eds.),*Proc.1 st Int. Conf. Cult.PenaeidPrawns/Shrimps.* SEAFDEC,IloiloCity,Philippines. pp. 47-64.

Primavera,J.H.(1988) Maturation, Reproduction and broodstock technology. In: *Biology and Culture of Penaeus monodon.* SEAFDEC,Iloilo City. pp 37-57.

*Primavera,J,H.and Yap,W.G.(1979) Status and problems of broodstock and hatchery management of sugpo (*Penaeus monodon*) and other penaeids. *Contr.No.4*, Aquacult. Dept., SEAFDEC,Iloilo, Philippines.

*Primavera,J.H.,Lim,C. and Borlongan,E.(1979) Feeding regime in relation to reproduction and survival of *Penaeus monodon. Philippine J.Biol.*,**8**:227-235.

Primavera,J.H., Young,T. and Reyes,C.D.L. (1982) Survival, Maturation, Fecundity and Hatching rates of unablated and ablated *Penaeus indicus* H.M.Edwards from brackishwater ponds. *Proc.Symp.Coast.Aquacult.*. Mar.Biol.Assoc.India.**1**: 47-54.

*Pudadera,R.A. and Primavera,J.H.(1981) Effect of light quality and eyestalk ablation on ovarian maturation in *Penaeus monodon. Kaliksan***10**:231-241.

Quackenbush,L.S.(1986) Crustacean endocrinology, A review. *Can. J.Fish.Aquat.Sci.*, **43**:2271-2282.

Quackenbush,L.S.(1989) Yolk Protein Production in the Marine shrimp *Penaeus vannamei. J.Crust.Biol.,*9:509-516.

Quackenbush,L.S.(1991) Regulation of vitellogenesis in Penaeid Shrimp. In:P.F. DeLoach,W.J.Dougherty and M.A.Davidson (eds.), *Frontiers of Shrimp Research.* Elsevier Science Publishers,Amsterdam. pp 71-81.

Quackenbush,L.S.(1992) Yolk Synthesis in the Marine Shrimp, *Penaeus vannamei. Comp. Biochem.Physiol.,*103(A):711-714.

Quackenbush,L.S. and Herrnkind,W.F.(1981) Regulation of molt and gonadal development in the spiny lobster,*Panulirus argus*: effect of eyestalk ablation. *Comp. Biochem.Physiol.,*69(A):523- 527.

Quackenbush,L.S.and Herrnkind,W.F.(1983) Partial characterization of eyestalk hormones controlling molt and gonad development in the spiny lobster, *Panulirus argus. J.Crust.Biol.,*13: 34-44.

*Quackenbush,L.S. and Keeley,L.L.(1987) Vitellogenesis in the shrimp *Penaeus vannamei. American Zool.,*27:810A (Abstract only).

Quinitio,E.T.and Millamena,O.M.(1992) Ovarian changes and female specific protein levels during sexual maturation of the white shrimp *Penaeus indicus. Bamidgeh,* 44:7-12.

Quinitio,E.T.,Hara,A.,Yamouchi,K. and Fuji,A.(1990) Isolation and characterization of vitellin from the ovary of *Penaeus monodon. Inv.Reprod.Dev.*17:221-227.

Quinitio,E.T.,Yamouchi,K.,Hara,A. and Fuji,A.(1991) Profiles of Progesterone and Estradiol like substances in the Haemolymph of Female *Pandalus kessleri* during an annual Reproductive cycle. *Gen.Comp.Endocrinol.*81:343-348.

*Raabe,M.(1982) *Insect neurohornes.* Plenum,New York 352 pp.

*Racek,A.A.(1973) Indo-west Pacific penaeid prawns of commercial importance. In: T.V.R.Pillay (ed.),*Coast.Aquacult.Indo-Pacific region.*Fishing News (Books) Ltd, London. pp.152-172.

Rajyalakshmi,T.,Ravichandran,P.,Pillai,S.M.and Mohanty,M.(1988) Induced maturation of *Penaeus monodon* in a recirculatory system. In: M.Mohan Joseph (ed.), *Proc.I st Indian Fish.Forum.* Asian Fish. Soc.,Indian Branch, Mangalore. pp 103-105.

Ramos,T.L.(1985) Induction to ovary maturation by ablation in the pink shrimp *Penaeus notialis*. In:Y.Taki,J.H.Primavera and J.A.Llobrera (eds.),*Proc.1 st Int.Conf. Culture Penaeid Prawns/Shrimps*. SEAFDEC, Iloilo City, Philippines. p.67 (Abstract only).

Rankin,S.M.,Bradfield,J.Y.and Keeley,L.L.(1989) Ovarian protein synthesis in the South American white shrimp, *Penaeus vannamei*,during the reproductive cycle. *Invert. Reprod.Dev.*, **15**:27-33.

Rao.P.V.(1968) Maturation and Spawning of the Penaeid Prawns of the Southwest Coast of India. *FAO Fish.Rep.*,**57**(2):285-302.

Rao,.P.V. and Kathirvel,M.(1973) On the Breeding of a Penaeid Prawn, *Metapenaeus dobsonii* in the Brackishwater Medium. *Indian J.Fish.*,**20**:228-230.

*Read,H.L. and Caulton,M.S.(1980) Changes in mass and chemical components during the moult cycle and ovarian development in immature and mature *Penaeus indicus* Milne Edwards. *Comp. Biochem.Physiol.*,**66**(A):431-437.

*Rengneker,P.V. and Deshmukh,R.D.(1968) Effect of eyestalk ablation on the ovarian growth of the marine crab *Scylla serrata* (Forskal). *J.Anim. Morphol.Physiol.*, **15**: 116-126.

*Rice,P.R. and Armitage,K.B.(1974) The influence of photoperiod on processes associated with molting and reproduction in the crayfish *Orconectes nais* (Faxon). *Comp.Biochem.Physiol.*,**47**(A): 243-259.

*Richardson,H.G.,Deecaraman,M and Fingerman.M.(1991) The effect of Biogenic amines on ovarian Development in the fiddler crab, *Uca pugilator*. *Comp. Biochem.Physiol.*,**99**(C):53-56.

Robertson,L.,Bray,W.and Lawrence,A.(1991) Reproductive Response of *Penaeus stylirostris* to Temperature Manipulation. *J. World Aquacult.Soc.*,**22**:63-71.

Rodriguez,A.(1981) Growth and Sexual Maturation of *Penaeus kerathurus* (Forskal,1775) and *Palaemon serratus* (Pennant) in salt ponds. *Aquaculture*, **24**:257-266.

*Rodriguez,L.M.(1979) A guide to the mass scale production of *Penaeus monodon* spawners. Aquacult.Dept.,SEAFDEC,Iloilo.(mimeo).

*Ruangpanit,N.,Maneewongsa,S.,Pechmanee,T. and Tanen,T. (1981) Induced ovarian maturation and reproduction of *Penaeus monodon* Fabricius by eyestalk ablation. *Ann.Rep.Natl.Inst.Coast. Aquacult.Fish.Dept.(Thailand)*. pp.82-106.

*Ruello,N.V.(1973) The influence of rainfall on the distribution of and abundance of the school prawn *Metapenaeus mcleayi* in the Hunder river region (Australia). *Mar Biol.*, **23**:221-228.

*Sambasivarao,S.,Jayalakshmi,K. and Sarojini,R.(1985) Influence of the steroidal hormones on gonadal maturation in marine juvenile prawn, *Metapenaeus affinis*. *Proc.I st Natl.Symp. Comp.Endocrinol.Inv.*. pp.74-77.

Santiago,A.C.,Jr.(1977) Successful Spawning of cultured *Penaeus monodon* Fabricius after eyestalk ablation. *Aquaculture,***11**: 185-196.

*Sarojini,R. and Gyananath,G.(1984) Effect of steroids on the ovarian maturation of the freshwater prawn, *Macrobrachium lamerrii*. *Adv.Bios.,***3**:75-82.

Sarojini,R. and Persis,B.(1988) Stimulation of Oogenesis in freshwater prawn *Caridina rajadhari* by Human Gonadotropin. *Comp.Physiol.Ecol.,***13**:60-63.

*Sarojini,R.,Jayalakshmi,K. and Sambasivarao,S.(1986) Effect of external steroids on ovarian development in freshwater prawn, *Macrobrachium lamerrii*. *J.Adv. Zool.***7**:50-53.

Sarojini,R.,Mirajkar,M. and Nagabhushanam,R.(1982) The site of sex pheromone production in the freshwater prawn, *Macrobrachium kistensis*. *Curr.Sci.*, **51**: 975-978.

Sarojini,R.,Mirajkar,M.S.and Nagabhushanam,R.(1985) Effect of Steroids on Oogenesis and Spermatogenesis of the freshwater prawn, *Macrobrachium kistensis*. *Comp. Physiol.Ecol.,***10**:7-11.

*Sarojini,R.,Nagabhushanam,R. and Fingerman,M.(1993) *In vivo* evaluation of 5-HT stimulation of the testis in the field crab,*Uca pugilator*:A presumed action on the neuroendocrine system. *Comp.Biochem.Physiol.,***106(C)**:321-325.

Sarojini,R.Nagabhushanam,R. and Fingerman,M.(1994) 5 Hydroxy tryptaminergic control of testis development through the androgenic gland in the red swamp crayfish, *Procambarus clarkii*. *Inv.Reprod.Dev.,***26**:127-132.

Sarojini,R.,Rao,S.S. and Lakshmi,K.J.(1990) Effect of Steroids (Estradiol and Estrone) on the ovaries of the Marine crab, *Scylla serrata. Comp.Physiol.Ecol.*15:21-26.

*Sasikala,S.L. and Subramoniam,T.(1987) The effect of steroid hormones on the vitellogenesis of freshwater field crab *Paratelphusa hydrodromous. Symp.Freshw. Aquacult.*,Nagarjuna Univ., Dec.21-24.

*Schlagman,A.,Lewinson,C.and Tom,M.(1986) Aspects of the reproductive activity of *Penaeus semisulcatus* de Haan along the southeastern coast of Mediterranean. *P.S.Z.N.I.Mar.Ecol.*,7: 15-22.

*Schniederman,A. and Gilbert,L.I.(1958) Substances with juvenile hormone activity in crustacea and other invertebrates. *Biol. Bull.*,115:530-535.

Seat,M.Y. and Carlos,M.H.(1994) Maturation, Spawning, Hatching and Larval rearing of *Penaeus indicus* from the Red Sea, Saudi Arabia. *Proc.Aquacult.Symp.Tech.and Invest.Opportunities* . Ministry of Agricult. and Water, King Abdulazis City of Science and Techn. and Riyadh Chamber of Commerce and Industry. Riyadh, Saudi Arabia. pp.390-399.

Silas,E.G.,Muthu,M.S.,Pillai,N.N. and George,K.V. (1982) Breeding of the Marine prawn *Metapenaeus dobsonii* in Brackishwater ponds. *J.Mar.Biol.Ass.India.*, 24: 147-149.

Simon,C.(1982) Large scale commercial application of penaeid shrimp maturation technology. *J.World Maricult.Soc.*,13:301- 312.

Skinner,D.M.(1985) Molting and Regeneration. In:D.E.Bliss and L.H.Mantel (eds.), *The Biology of Crustacea.*Academic Press, New York.IX:43-145.

Snedecor,G.W. and Cochran,G.(1968) *Statistical methods.* Oxford & IBH Publishing Co.Ltd.,New Delhi. 595 pp.

*Souty,C. and Picaud,J.L.(1984) Effet de l'injection de une gonadotropine humaine sur la synthe'se et la vitellogenin pa le tissue adipaux du crustace' isopode marin, *Idotea balthica basteri. Gen.Comp.Endocrinol.*,54:418-421.

*Souty,C.,Besse,G. and Picaud,J.L.(1982) Ecdysone stimulation rate of vitellogenin release in haemolymph of the terrestrial crustacean isopod *Porcellio compressa.J.Exp.Zool.*,240: 203- 209.

*Soyez,D.,Le caer,J.P.,Noel,P.Y. and Rossier,J. (1991) Primary structure of two isoforms of the vitellogenesis inhibiting hormone from the lobster,*Homarus americanus.Neuropeptides*, **20**:25-32.

*Stephens,G.J.(1952) Mechanisms regulating reproductive cycle in the crayfish, *Cambarus* I.The female cycle. *Physiol.Zool.*,**25**: 70-84.

Subrahmanyam,C.B.(1963) A note on the annual reproductive cycle of the prawn, *Penaeus indicus*(M.Edw.) of Madras coast. *Curr. Sci.*,**32**:165-166.

Subrahmanyam,C.B.(1965) On the Reproductive cycle of *Penaeus indicus* (M. Edw.). *J.Mar.Biol.Ass.India*,**7**: 284-290.

Subramoniam,T. and Keller,R.(1993) A New Look at the Endocrine Regulation of Egg Maturation in the Decapod Crustaceans.*Curr. Sci.*,**65**:619-622.

Takayanagi,H., Yamamoto,Y. and Takeda,N.C. (1986a) An ovary stimulating factor in the freshwater shrimp, *Paratya compressa. J.Exp.Zool.*,**240**:203-209.

Takayanagi,H.,Yamamoto,Y. and Takeda,N.C.(1986b) Ovary stimulating pheromone in the freshwater shrimp,*Paratya compressa. J.Exp.Zool.*,**240**:397-400.

*Taketomi,Y.and Kawano,Y.(1985) Ultrastructure of the mandibular organ of the shrimp, *Penaeus japonicus*, in untreated and experimentally manipulated individuals. *Cell Biol. Intern. Rep.*,**9**:1069-1074.

Tan-Fermin,J.D.(1991) Effects of unilateral eyestalk ablation on ovarian histology and oocyte size frequency of wild and pond reared *Penaeus monodon* (Fabricius) broodstock. *Aquaculture*, **93**:77-86.

Tan-Fermin,J.D. and Pudadera,R.A.(1989) Ovarian Maturation stages of the wild giant prawn, *Penaeus monodon* Fabricius. *Aquaculture*,**77**:229-242.

*Tcholakian,R.K. and Eik-Nes,K.B.(1971) Steroidogenesis in the crab,*Callinectes sapidus. Gen.Comp.Endocrinol.*,**17**:115-124.

Tensen,C.P., Jannsen, K.P.C. and Van Herp,F. (1989) Isolation, characterization and physiological significance of crustacean hyperglycemic factors from the sinus gland of the lobster,*Homarus americanus*(Milne Edwards). *Intern.J.Inv.Reprod.Dev.*,**16**: 155-164.

*Teshima,S.and Kanazawa,A.(1971a) Production of ketotestosterone and other steroids by the sliced ovaries of, *Portunus trituberculatus*. *Bull.Jap.Soc.Sci.Fish.*,**37**: 246-249.

*Teshima,S. and Kanazawa,A.(1971b) Bioconversion of progesterone by the ovaries of the crab,*Portunus trituberculatus.Gen.Comp. Endocrinol.*,**17**:152-157.

*Teshima,S. and Kanazawa,A.(1971c) *In vitro* bioconversion of progesterone to 17-hydroxy progesterone and testosterone by sliced testis of crab,*Portunus trituberculatus. Bull.Jap.Soc. Sci.Fish.*,**37**:523-528.

*Teshima,S. and Kanazawa,A.(1973) Metabolism of desmatids in the prawn *Penaeus japonicus. Mem.Fac.Kagoshima Univ.*,**22**:15-19.

*Teshima,S. and Kanazawa,A.(1983) Variation in lipid components during ovarian maturation of the prawn. *Bull.Jap.Soc.Sci. Fish.*,**49**:957-962.

Teshima,S. and Kanazawa,A.(1986) Nutritive value of sterols for the juvenile prawn. *Bull.Jap.Soc.Sci.Fish.*,**52**:1417-1422.

Teshima,S.,Kanazawa,A.,Koshio,S.and Horinouchi,K.(1988a) Lipid metabolism in destalked prawn *Penaeus japonicus* : Induced maturation and accumulation of lipids in the ovaries.*Nipp. Suis.Gakk.*,**54**:1115-1122.

Teshima,K.,Kanazawa,A.,Horinouchi,K.and Koshio,S.(1988b) Lipid metabolism in destalked prawn *Penaeus japonicus:*Induced maturation and transfer of lipid reserves to the ovaries. *Nipp. Suis.Gakk.*, **54**:1123-1129.

Teshima,S.,Kanazawa,A.,Koshio,S. and Horinouchi,K.(1989) Lipid metabolism of the prawn, *Penaeus japonicus* maturation: variation in lipid profile of the ovary and hepatopancreas. *Comp.Biochem.Physiol.*,**92(B)**:45-49.

*Thampy,D.M. and John,J.A.(1972) The androgenic gland of shrimp *Palaemon dayanus. Mar.Biol.*,**4**:285-288.

*Tobe,S.S.,Young,D.A.,Khoo,H.W. and Baker,F.C.(1989) Farnesoic acid as a major product of release from crustacean mandibular organ *in vitro. J.Exp.Zool.*, **249**: 165-171.

*Toullec,J.-Y. and Wormhoudt,A.V.(1987) Variations quantitatives durant de cycle d' intermue de peptides apparent es a pheromone de croissance humane chez *Palaemon serratus* (Crustace,Decapode).*C.R.Acad.Sci.,Paris*,**350**:265-269.

Trujillo,L.R. and Primavera,J.H.(1986) Induced Maturation in ablated *Penaeus notialis* and *Penaeus schmitti*.In:J.L.McClean, W.B.Dizon and L.Vittosillos (eds.), *Proc. First Asian Fish. Forum*. Asian Fish. Soc., Manila, Philippines. pp.697-700.

*Tsukimura,B.(1988) The role of presumptive mandibular organ secretion in ovarian growth of the shrimp,*Penaeus vannamei*. *Ph. D.Dissertation*, Univ.Hawaii,Hawaii. 90pp.

Tsukimura,B. and Kamemoto,F.I.(1988) Organ culture assay of the effect of putative reproductive hormone on immature penaeid ovaries. *J.World Aquacult.Soc.*,19:288 (Abstract only).

Tsukimura,B. and Kamemoto,F.I.(1991) *In Vitro* Stimulation of Oocytes by Presumptive Mandibular Organ Secretions in the shrimp, *Penaeus vannamei*. *Aquaculture*,92: 59-66.

*Tsukimura,B.,Tanji,T.M. and Kamemoto,F.I.(1986) Sinus gland activation of cyclic GMP in the mandibular organ of *Homarus americanus*. *American Zool.*,26:91A (Abstract only).

*Tsukimura,B.,Markin,M,Frinsko,M.and Borst,D.W.(1989) Measurement of methyl farnesoate (MF) in crustacean hemolymph. *Amer. Zool.*,29:49A (Abstract only).

*Tuma,D.J.(1967) A description of the development of the primary and secondary sexual characters in the banana prawn : *Penaeus merguiensis* de Man (Crustacea: Decapoda: Penaeidae). *Australian J.Mar.Freshw.Res.*,18:73-88.

*Villaluz,D.K.,Villaluz,A.,Landrera,B., Shaik,M. and Gonzaga,A. (1969)Reproduction larval development and cultivation of sugpo (*Penaeus monodon* Fabricius). *Philippine J.Sci.*, 98: 205-236.

*Vincente,H.J.,Valdez,F.M. and Valdez,L.S.(1979) Land based maturation and spawning of *Penaeus monodon* Fabricius in MSU- IFRD and its future research aspects. *Mindanoo State Univ.Inst.Fish.Res.Dev.Rep.*. pp.115-123.

Walker,T.(1994) Improving the Return from prawn spawners : Research shows the way. *Austasia Aquaculture*,8(4):7-9.

*Watanabe,T.(1985) Importance of broodstock nutrition for faster development of aquaculture. In:C.B.Cowey,A.M.Mackie and T.G.Bell (eds.),*Nutrition and feeding in fish*. Acad.Press. London. pp.395-414.

Waterman, J.H. (1961) Light sensitivity and vision. In: J.H. Waterman (ed.),*The physiology of crustacea*.Acad.Press.New York.7II:1-64.

*Wear,R.G. and Santiago,Jr.,A.(1976) Induction of maturity and spawning in *Penaeus monodon* Fabricius by unilateral ablation (Decapoda:Natantia). *Crustaceana*,31: 218-220.

Webster,S.G. and Keller,R.(1986) Purification, characterization and amino acid composition of the putative molt inhibiting hormone (MIH) of *Carcinus maenas* (Crustacea,Decapoda). *J.Comp. Physiol.*,**156(B)**:617-624.

*Weitzman,M.C.(1964) Ovarian development and moulting in the tropical land crab, *Gecarcinus lateralis* (Fre'mivilles). *American Zool.*,4:329-330.

Wickins,J.F. and Beard,J.W.(1974) Observations on the breeding and growth of giant freshwater prawn *Macrobrachium rosenbergii* (De Man)in the laboratory. *Aquaculture*, 3:159-174.

Wyban,J.A., Lee,C.S., Sweeney,J.N. and Richards,W.K.,Jr. (1987) Observations on development of a maturation system for *Penaeus vannamei*. *J.World Aquacult. Soc.*, 18:198-200.

Xu,X.L.,Ji,W.J.,Castel,J.D. and O'Dor,R.K.(1994) Influence of dietary lipid sources on fecundity,egg hatchability and fatty acid component of Chinese prawn (*Penaeus chinensis*) broodstock. *Aquaculture*,119:359-370.

Yano,I.(1984) Induction of rapid spawning in kuruma prawn, *Penaeus japonicus*,through unilateral eyestalk enucleation. *Aquaculture*,40:265-268.

Yano,I.(1985) Induced Ovarian maturation and spawning in greasyback shrimp, *Metapenaeus ensis* by Progesterone. *Aquaculture*, 47:223-229.

Yano,I.(1987) Effect of 17-a- Hydroxy Progesterone on vitellogenin secretion in kuruma prawn, *Penaeus japonicus*. *Aquaculture*,61:49-57.

Yano,I (1988) Oocyte Development in the kuruma prawn *Penaeus japonicus*. *Mar.Biol.*,99:547-553.

Yano,I.(1992a) Hormonal Control of Reproductive Maturation in Penaeid shrimp. *NOAA Tech.Rep.*. NMFS,106:7-14.

Yano,I.(1992b) Effect of Thoracic Ganglion on Vitellogenin secretion in kuruma prawn, *Penaeus japonicus.Bull.Natl.Res. Inst.Aquacult.*,21:9-14.

*Yano,I. and Chinzei,Y.(1991) Effect of human chorionic gonadotropin and unilateral enucleation on vitellogenesis induction and ovary development in kuruma prawn, *Penaeus japonicus.* In: C.C.Justo (ed.), *The aquacult.Shrimps,Prawns and Crayfishes in the World: Basics and Techn.*. Tokyo. pp.365-370.

Yano,I. and Wyban,J.A.(1987) Induced maturation and spawning of *Penaeus vannamei.* *J.World Aquacult.Soc.*,18:29A (Abstract only).

Yano,I. and Wyban,J.A.(1992) Induced Ovarian Maturation of *Penaeus vannamei* by injection of Lobster Brain Extract. *Bull. Natl.Res Inst.Aquacult.*,21:1-7.

Yano,I.and Wyban,J.A.(1993) Effect of Unilateral Eyestalk ablation on spawning and hatching in *Penaeus vannamei. Bull.Natl. Res.Inst.Aquacult.*,22:21-25.

Yano,I.,Kanna,R.A.,Oyama,R.N. and Wyban,J.A.(1988a) Mating behaviour in the penaeid shrimp *Penaeus vannamei.Mar.Biol.*, 97:171-175.

Yano,I.,Tsukimura,B.,Sweeney,J.A.and Wyban,J.A.(1988b) Induced Ovarian Maturation of *Penaeus vannamei* by Implantation of Lobster Ganglion. *J.World Aquacult.Soc.*, 19:204-209.

*Yashiro,R.(1989) Biochemical,immunonological and histological studies on ovarian maturation and rematuration of *Penaeus monodon* Fabricius. *Ph.D.Dissertation.* Univ.Philppines, Vysyas.

*Young,J.(1959) Morphology of the white shrimp *Penaeus setiferus* (Linnaeus,1758). *Fish.Bull.*,59:1-168.

Young,N.J.,Quinlan,P.T. and Goad,L.T.(1992) Progesterone metabolism *in Vitro* in the Decapod Crustacean,*Penaeus monodon. Gen.Comp.Endocrinol.*,87:300-311.

Young,N.J.,Webster,S.G.and Rees,H.H.(1993) Ecdysteroid profiles and vitellogenesis in *Penaeus monodon* (Crustacea:Decapoda). *J.Invert.Reprod.Dev.*,24:107-118.

Zhang,D.(1990) Overwintering and Sexual Maturation of *Penaeus pencillatus* Alcock in an outdoor earth pond. *Aquaculture*,86: 327-331.

Zukowska-Arendarczyk,M.(1981) Effect of Hypophysial Gonadotropins (FSH and LH) on the ovaries of the sand shrimp *Crangon crangon* (Crustacea:Decapoda). *Mar.Biol.*,**63**:241-247.

*Not consulted in original

INDUCED MATURATION OF *PENAEUS INDICUS* USING EXOGENOUS HORMONE

By

JOHNSON D' CRUZ, B.F.Sc.

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement for the degree

MASTER OF FISHERIES SCIENCE

Faculty of Fisheries

Kerala Agricultural University

DEPARTMENT OF AQUACULTURE

COLLEGE OF FISHERIES

PANANGAD, COCHIN

1995

VIII ABSTRACT

The ovarian maturation and spawning of broodstock shrimp play a major role in the development of commercial level seed production of this valuable crustacean. The already proven technology of induced maturation of penaeids in captivity, viz. Unilateral Eyestalk Ablation (UESA) has many negative effects which prompted scientists to explore alternative ways of induced maturation like hormone administration. The hormone progesterone alone and in combination with tocopherol were tested for their effectiveness in bringing about ovary maturation and spawning in pond reared *Penaeus indicus*.

Progesterone and tocopherol dissolved in ethyl alcohol were injected at three test concentrations of 0.05 ug, 0.10 ug and 0.20 ug/g body weight. For the preparation of combination test solution the dissolved progesterone and tocopherol were mixed in the ratio of 7:3 (V/V). Control shrimps were given alcohol treatment at respective concentrations.

The results of the experiment conducted for a period of after 45 days, revealed that the overall survival is not affected (89 %) and that progesterone singly or in combination with tocopherol is capable of giving stimulation for ovarian development in *P.indicus* . Though there was not much observable difference in ovarian development by external viewing, both progesterone alone and in combination with tocopherol at higher dosages have yielded significant GSI difference and ovarian growth, compared with the ethanol treated controls, when the ovaries were dissected out and subjected to detailed study. The percentage somatic growth which was high for control shrimps in contrast to the hormone

treated animals having more ovarian growth, suggests an antagonism between these two energy demanding processes.

The failure in producing an external visible change in ovarian development, in comparison to eyestalk ablation (ESA) treatment may be due to the existence of the gonad inhibiting hormone (GIH) produced in the eyestalk which would have blocked/suppressed further development of ovary. It is likely that if a GIH inhibitor is also administered along with steroid hormones or tocopherol the stimulus given by them may result in full ovarian development in penaeid shrimp.

-