STUDIES ON THE REPRODUCTIVE PHYSIOLOGY AND BREEDING BIOLOGY OF CULTIVABLE SPECIES OF GROUPER *EPINEPHELUS TAUVINA* (FORSSKAL)

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BY

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CERTIFICATE

This is to certify that the thesis entitled **"Studies on the reproductive physiology and breeding biology of the cultivable species of grouper *Epinephelus tauvina* (Forsskal)"** is an authentic record of research work carried out by Mrs.Gracy Mathew under my guidance and supervision in partial fulfillment for degree of Doctor of Philosophy of Cochin University of Science & Technology under the faculty of Marine Sciences.

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DECLARATION

I, hereby declare that this thesis entitled "**Studies on the reproductive physiology and breeding biology of the cultivable species of grouper *Epinephelus tauvina* (Forsskal)**" has not previously formed the basis for the award of any degree, associate– ship, fellowship or any other similar title or recognition.

Gracy Mathew

March 2008
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PREFACE

According to the projections given by the FAO, in order to maintain the average per capita supply of 17.2kg, it will be necessary to produce nearly 70 million metric tonnes of food fish by 2020, from both capture fisheries and culture fisheries together. This clearly points out that the burden has to be borne by aquaculture as the marine fish production from capture has reached a plateau limit of around 2.7 million metric tonnes in the last decade. India with its enormous potential for sea farming, should harness this to the maximum for increasing the fish production. The marine and brackish water fishes make up an important component in terms of production and value. The development of large and affluent markets for live reef fish in Hong Kong and Southern China has increased pressure on wild stock resources and has led to indiscriminate fishing including destructive fishing methods such as dynamite and cyanide fishing, resulting in the depletion of the natural stocks to an alarming level. Aquaculture of high-value finfish species, such as groupers, is an industry of increasing importance throughout the Asia-Pacific region.

The estuarine grouper *Epinephelus tauvina* is one of the species used foremost in mariculture for the last two decades in the Southeast Asian countries, because of its high market demand, fairly fast growth rate, good food conversion ratio, disease resistance and tolerance to crowding. The growing demand for quality seed coupled with uncertainty of availability from nature at the appropriate time in required quantities has prompted research on problems connected with seed production. Attempts on breeding and hatchery production of seeds of groupers are progressing actively in many countries world around. But a number of lacunae still remain for optimizing

commercial seed production. The significance of developing a technology for brood stock development and breeding in captive condition is imperative in ensuring seed availability at the required time. To develop a genetic programme to improve the population characteristics, it is necessary to control maturation and reproduction in captivity. In order to proceed with controlled reproduction or artificial means of reproduction to produce good quality eggs, an in-depth knowledge of the reproductive biology, various stages of gonad maturation, physiology of spawning etc. should be known. The present study envisages the objectives of obtaining a detailed picture of the physiology of reproduction ie. the oogenesis and spermatogenesis in the protogynous estuarine grouper *E. tauvina* .

The results of the entire studies are embodied in the present thesis. It starts with a general introduction, covering the present status of our knowledge on the subject and emphasizes the importance of the subject of investigation. Subsequent to this, there are five separate chapters, covering different aspects such as the female reproductive system and the process of oogenesis, hermaphroditism, sex-inversion, male reproductive system and spermatogenesis, breeding biology including brood stock development, fecundity, spawning, incubation and hatching etc. The sixth chapter is on grouper culture, and there is also a chapter that pertains to the taxonomical aspects and distribution of commercially important species of groupers, including *E. tauvina.* Each chapter begins with an introduction, followed by materials and methods, results and discussion. Introductory part of each chapter highlights the importance of the particular aspect of study and also reviews literature pertaining to it. Materials and methods explain the materials and techniques used as well as the procedures adopted for data analysis. The data obtained are presented in the results with necessary

figures and tables. Each chapter is concluded with a discussion about the salient findings of the study.

Chapter two deals with the phylogenic taxonomy of groupers as well as taxonomic status and distribution of six commercially important species. This study was carried out because phylogenetic systematics among the groupers are poorly known and relationships among the genera have been the subject of much disagreement. The salient diagnostic characters of each species along with the geographical distribution are given.

Control of reproduction of the candidate species is one of the most important aspects of aquaculture management; for ensuring high quality seed production and genetic improvement of stock. For carrying out artificial means of reproduction aquaculturists must be fully aware of the gonadal maturation stages, spawning seasons and status of the brooders during spawning. The third chapter on the female reproductive system and development of the ova explains the general anatomy of the various stages of the ovary; the methods of determining the reproductive cycle and the developmental changes in the gonads through microscopic observations. In *E. tauvina,* in fishes measuring 38-40cm in total length, the females can be distinguished by the presence of pinkish, translucent, ribbon-like strands of gonadal tissue. Ovarian maturation was classified into six developmental stages mainly based on the changes in size, shape, colour, diameter of oocyte and on microscopic structure of the ova. The production of egg, well equipped with the necessary reserve food for the developing embryo, occurs through the processes taking place in the germ mother cells of the ovary. Clear knowledge of the precise stage of gonadal maturation is highly essential for the

selection of female spawners for breeding and seed production. This process of oogenesis has been studied by the use of histological methods.

In the present study, for captive spawning of *E. tauvina* which is a protogynous hermaphrodite, male brooders were developed by transforming female fish to male. Sex reversal was effected by oral administration of 17∝ methyl testosterone (MT) through trash fish used as feed . Chapter four deals with sex inversion through hormone application, description of male reproductive organ and spermatogenesis using histological and cytological criteria . The progressive development of male tissues and transformation to the male gonad is sequentially followed with increase in the dosage of the male hormone. Spermatogenesis was found to involve progressive reduction in cytoplasmic volume and condensation of chromatin matter.

In the next chapter breeding biology of the estuarine grouper *E.tauvina* is dealt in detail. Natural spawning of the species by environmental manipulation under captive condition is given. Effect of lunar periodicity on the natural breeding of grouper is also elucidated. Fertilization rate, incubation and hatching rate of the buoyant, viable eggs are dealt with in this chapter. The Sixth chapter is on the farming or culture of groupers. The global scenario and the current methods of grouper farming world over are summarized here. Experimental culture of groupers carried out in the onshore recirculating system using different stocking densities are also elucidated. The last chapter is a summary of conclusions, which constitute the overall findings of the study carried out and conclusions drawn from it. Literature cited in the entire study is listed under the reference given at the end of the thesis.

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STUDIES ON THE REPRODUCTIVE PHYSIOLOGY AND BREEDING BIOLOGY OF THE CULTIVABLE SPECIES OF GROUPER *EPINEPHELUS TAUVINA* (FORSSKAL).

Contents

Chapter 1.

Introduction.

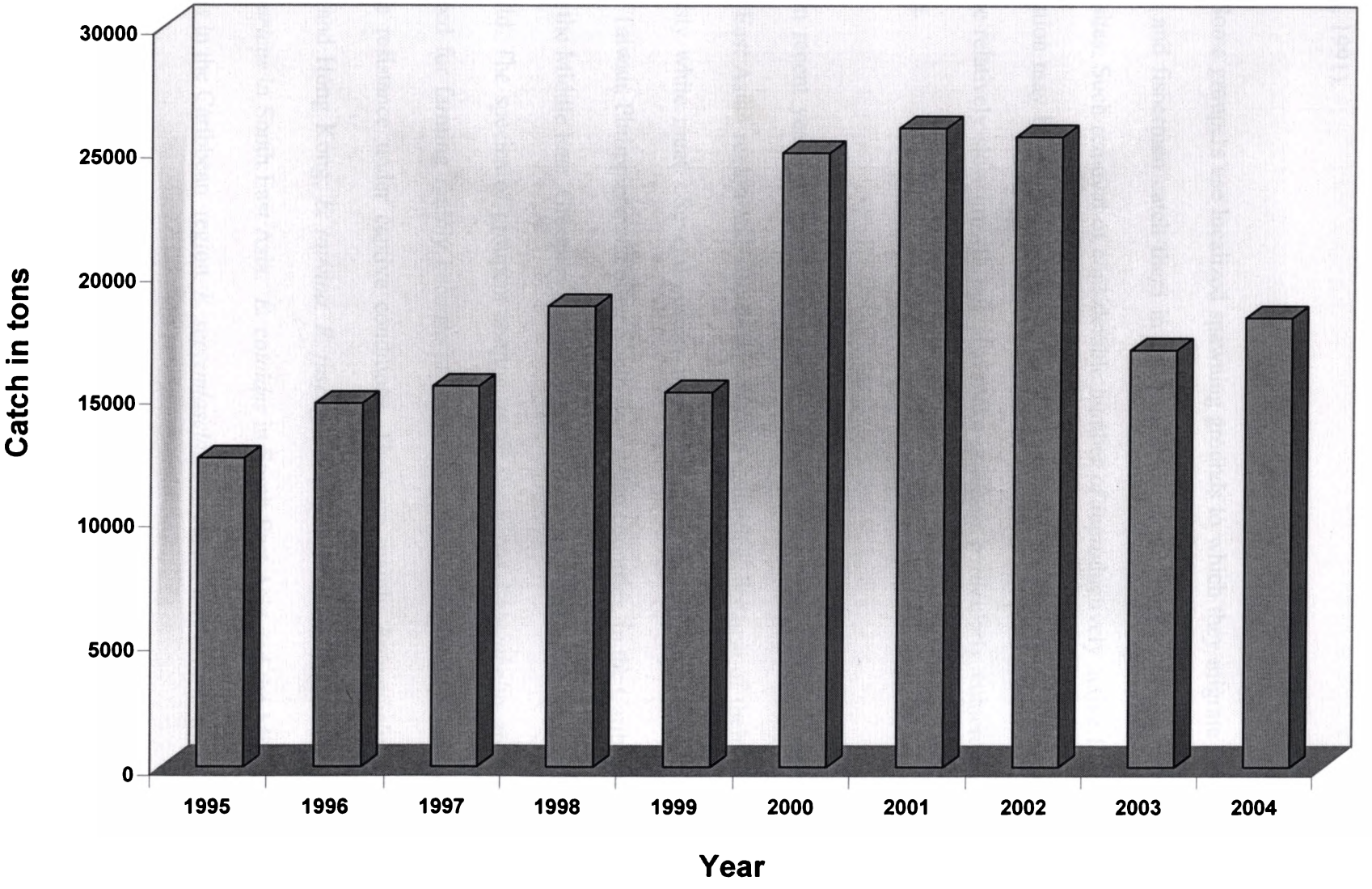
The marine fish production in India on reaching a threshold limit of around 2.7 million metric tonnes in the last decade is now at standstill. India with its enormous potential for sea farming, could harness this to the maximum for increasing the fish production. Though resource enhancement through aquaculture has made tremendous growth in the freshwater sector, the strides taken in the marine sector are very few except for shrimp farming. Expansion of fish culture is dependent upon regular and predictable supplies of fingerlings for stocking, while the economic success of the whole operation depends on improved growth performance and efficient food conversion by the fish. Among the many species of cultivable food fishes and shellfishes in the brackish water, coastal and enclosed marine habitats, fin fishes like groupers are highly promising candidate species for augmenting fish production. In many South East Asian countries groupers are considered a viable substitute for commercial culture in old shrimp farms (Anon,1999).

Groupers belonging to the family Serranidae and subfamily Epinephelinae comprising a number of genera, are carnivorous fishes distributed in most of the tropical and subtropical seas around the world. Thirty-eight species of groupers are known (James *et al.*, 1996) from the seas around India, but only a few contribute to the

commercial fishery. Most of the species belonging to this group inhabit the rocky grounds and the coral reef areas, while a few prefer the sea grass beds and the muddy and sandy bottoms in the inshore regions in the Palk Bay and Gulf of Mannar, the Gulf of Kutch, Malwan, the lagoons of Lakshadweep islands (Bensam, 1993) . These fishes are abundant in the vast stretches of rocky banks along the west coast and Wadge Bank; in the coral reef areas distributed along the east coast and around the Andaman islands, where the grounds are unsuitable for fishing using trawl nets or gill nets. Juveniles of a few species commonly occur in the nearshore areas, river mouths and lower reaches of estuaries. Except for the spawning aggregations most of the species are solitary. They are sedentary in character and strongly territorial (Bullock *et al.*, 1992, Morris *et al.*, 2000). All of them are top predators preying upon other fishes and invertebrates. The slow growth rate, late reproduction, large size and long life spans make groupers vulnerable to over-exploitation.

As early as 1916, Hornell has reported on the existence of good line fishing grounds for groupers and snappers along the Travancore coast, followed by many reports on the existence of a seasonal kalava fishery along the EEZ of the South West and South East coasts of India (Menon and Joseph 1969; Silas 1969; Joseph 1986; Joseph *et al.*, 1987; Mohan 1983; Sivaprakasam 1986; Somavanshi and Bhar 1984; Sulochanan and John 1988; Kasim *et al* ., 1987; Mathew and Venugopalan 1990, Mathew 1994 ; Mathew *et al* .,1996; Bennet and Arumugham 1994; James *et al.*,1994; Lazarus *et al.*, 1994, Prabhu 1954). The estimated annual average landings of groupers during 1995-2004 are 18,775 t (Fig.1). Almost the entire catch is taken from the inshore waters upto about 100m depths.

Fig.1. All India Grouper Catch 1995-2004



The potential yield of the groupers in the Indian EEZ is estimated to be around 54, 600 t (Anon. 1991).

Some groupers use localized spawning grounds to which they migrate from distant places and fishermen catch them in large numbers in the brief spawning period from these sites. Such removal of considerable number of reproductively active fish from the population may be detrimental to sustained yields from the fishery. The site specificity and the relatively slow growth rate also make groupers particularly vulnerable to over-fishing.

In recent years groupers are relished as high value food fishes especially in the South East Asian region and also in the western countries because of their soft, tender and tasty white meat. Several species of groupers are farmed commercially in Hong Kong, Taiwan, Philippines and other South East Asian countries, in the Caribbean islands and in the Middle East. Groupers are cultured mainly by growing juveniles captured from the wild. The species of groupers coming under the genus *Epinephelus* are commonly preferred for farming mainly because of their fast growth, adaptability and fairly high disease resistance under captive conditions. They are mainly *Epinephelus akaara* in Japan and Hong Kong; *E. tauvina, E. fuscoguttatus, E.salmoides, E.lanceolatus* and *E. malabaricus* in South East Asia, *E. coioides* in South East Asia and the Middle east, *E. striatus* in the Caribbean region, *E. septemfasciatus* in Japan, *E.areolatus* in Hong Kong and *Plectropomus leopardus* in South East Asia. Groupers are the most intenseively-exploited group in the live fish trade. Live trade of groupers is a lucrative venture in

Hong Kong, exported from many countries including India where these fishes fetch very high price of upto 50 US $ per Kg.

Development of grouper culture is one of the most important aquaculture targets in the tropics. High demand for these reef fishes in the international markets has led to indiscriminate fishing including destructive fishing methods such as dynamite and cyanide fishing, resulting in the depletion of the natural stocks to an alarming level. Aquaculture is perceived as the only alternative to the declining fishery from the natural grounds. Groupers have many advantageous features as candidate species for aquaculture. They are a hardy group that can tolerate wide fluctuations in salinity and temperature and can also be grown both in grow-out culture as well as in hatchery phase. They have fairly good food conversion ratio and fast growth rate and can even be trained on palletized feed making their culture economical. Most species of groupers are tolerant to crowding and to a great extent they are resistant to disease, pollution, stress etc. Groupers attain marketable size in 6-7 months of culture, well before reaching size at first maturity. They can be bred in captivity, have good frequency of spawning, high fecundity and mass larval rearing is also possible and also were found suitable substitute for commercial culture in old shrimp farms.

The single most important desideratum for successful mariculture activity is the availability of pure seed of uniform age, size and quality, free of diseases, parasites and pests. These strict requirements are seldom fulfilled where seed is collected from rivers, estuaries or other natural sources. The non-availability of sufficient quantity of seeds

from the natural grounds at the right time for farming purposes is another bottleneck in the progress of grouper aquaculture. For a successful aquaculture industry, timely availability of required quantity of pure quality seeds is highly essential. This is possible only by developing viable and sustainable technology for spawning and hatchery production of seeds. Attempts on breeding and hatchery production of seeds of groupers are progressing actively in many countries world around.

Almost all the species of groupers are well distributed in the Indo-West Pacific from western Indian Ocean from Madagascar, Sri Lanka, Eastern Indian Ocean, eastward to China, Philippines and Japan. *Epinephelus tauvina* is one of the most commonly occurring species along both the west and east coasts of India. Juveniles of this species are available at certain specific localities in the near shore region where sea grass beds are abundant and also near the mouths of estuaries. This species is commonly cultured commercially in South East Asia for more than a decade now.

Groupers are an example of hermaphroditism where one sex is followed by the other, in the life time of the animal – protogynous hermaphroditism- is known to occur in many species of groupers. (Smith 1965, Brusle and Brusle 1975, Bouain and Siau 1980, Shapiro 1987, Tan and Tan 1974, Yashiro *et al.*, 1993). Most species of groupers mature within two to six years (Tucker 1998). They mature as females early in their life, but reversing into functional males at older age. The success of spawning of groupers and production of seeds for aquaculture depends largely on the availability of mature brooders of both male and female in healthy condition and in sufficient numbers. This

includes development of male brooders and improvement of the quality of female brooders. Adult males of groupers are larger in size; often exceed one meter in length, fewer in number and occur in the deeper seas. Broodfish can be caught from the wild and made to spawn after conditioning them in captivity. Lack of improved methods of catching the breeders from the deeper grounds, stress caused by sudden change in environment etc. are major constraints experienced in capturing spawners from the wild and maintaining them. Male brooders for spawning purposes can be developed by artificially transforming female groupers to sexual males. Information on the onset and process of natural sex change, under captivity is scanty in grouper species. Spawning of groupers is therefore often resorted to by artificially transforming female fish to male and developing them as male spawners.

Histological changes that take place in the gonad during the process of sexual development and sex change has been studied for a number of species by several workers. Some species as a rule change from female to male with age, but some others might change only if there is a shortage of males and the relationship between sex change and age composition is not clearly understood yet in many species. Smith (1965) described the occurrence of protogynous hermaphroditism in many species of Epinephelus, and pointed out that there are three patterns of hermaphroditism in serranids: the *Serranus* type, the *Rypticus* type and the *Epinephelus* type. The *Epinephelus* and its allies have gonads in which the male tissue is present throughout the germinal epithelium lining the central lumen of the gonad. Here the male tissue becomes functional only after the female tissue has ceased to function. In the genus *Rypticus*, an

intermediate type of gonad in which scanty male tissues are present in the lower part but is also found intermixed with the female tissue. The protogynous *Chelidoperca hirudinacea* is of this type (Reinboth,1967). Reinboth (1963) has demonstrated that protogynous hermaphroditism in the Serranid *Sacura margaritacea* occurs by degeneration of ovarian tissue after spawning. Sex reversal among groupers has been described by many workers like Warner (1975), Warner *et al.*, (1975), Webb and Kingsford (1992) and Tessy (1994). Recently, Frisch (2004) has reviewed sex change and gonadal steroids in sequentially hermaphroditic teleost fishes.

A broad overlap of length distribution is encountered in many species suggesting that there is no close correlation of age or size with sexual transition . Males were not found among smaller size groups and the proportion of males increased with increasing age in the case of mature fish. Brusle and Brusle (1975) observed all young fishes to be females in *E.aneus* and *E.guaza* – the first functional activity being oogenetic and that males occur among largest individuals after sex reversal. All the young fishes of *E.chlorostigma* in the length range from 14 to 30 cm collected from the Red Sea were found to be females but a broad overlap of sexes was encountered among those in the length group 30 to 56cm, while at lengths more than 56cms all the examined fishes were found to be males (Ghorab *et al.*, 1986). Smith (1967) postulated that sex reversal takes place in different individuals at different sizes and ages and that sexual succession is a prolonged and continuous process for the population. He also interpreted that 5% of the individuals change before sexual maturity and that half the females do not change at all in their lifetime.

Our knowledge about the cause of sex reversal among groupers is very little, mostly based on observations on natural populations. In *E.tauvina*, the transition of sex from female to male begins at the age of 7 years (Tan and Tan 1974), and the proportion of males thereafter increased (Chen *et al.*, 1977; Chao and Lim, 1991). In the dusky grouper (*E.marginatus*) spontaneous sex inversion rarely occurs in the captive stock until 14 years (Glamuzina *et al.*, 1988) and such long term husbandry and maintenance of broodstock are time-consuming and tedious. Consequently, the male broodstock for propogation are generally obtained by means of induced sex change at relatively early age (Yeh *et al.*,2003). Concomitant with the advance in fin fish breeding technology, there has been significant progress in grouper breeding studies. The success of fish spawning depends on the availability of sufficient numbers of mature female and male brooders in healthy condition. Methodologies for inducing sex change have been reported by Kuo *et al.*, 1988, Chao and Chow, 1990, Chao and Lim 1991 and Quinitio *et al.*, (1997). Spermatogenesis has been induced and maintained in many species of fish using a variety of hormones such as human chorionic gonadotropin (HCG), Methyl testosterone (MT) or combinations of HCG and testosterone propionate (TP), ovine prolactin (LtH), and LH-RH-a. The success and time duration required for completion of sex change depend on the type and dosage of hormones and the manner of hormone administration. Shehadeh *et al.*, (1973) demonstrated that 17α methyl testosterone or HCG can be used to induce spermatogenesis and spermiation in mullets during spawning and prespawning season and that 17α methyl testosterone was found to be more effective than HCG.

Ukawa *et al.*, (1966) described the spawning behaviour of *E.akaara* as the interaction between a single male and female and that they spawned between 1530 and 1630 hrs in the culture ponds. There is no coupling during spawning and fertilization is external. Okamura *et al.*, (2002) gives an account of the spawning behaviour and artificial fertilization of captive reared red grouper *E. akkara* in Japan. Shapiro *et al.*, (1993) described the size, composition and spatial structure of annual spawning aggregations of the red hind *E. guttatus*. Under natural conditions, groupers aggregate during spawning season in the Bermuda and Bahamas in the Caribbean Sea. Spawning aggregations of *E.guttatus* and *E.striatus* were described by Colin *et al.*, (1987) and Alfonso and William (1996). Colin (1992) also described the spawning aggregations of Nassau grouper *E. guttatus* along the South Long Island with peak spawning season in December and January months. Domeier and Colin (1997) have reviewed spawning aggregations of tropical reef fish. Bardach (1958) described that spawning season of most species of groupers in the Bermuda coast extends from late April to late August. Erdman (1956) reported that *E.guttatus* spawn in January. Randall and Brock (1960) observed that peak spawning activity of certain Indo-Pacific species of groupers occurred a few days before full moon. Moe (1969) reported that in Gulf of Mexico, *E. morio* spawned from March to July with peak spawning activity during April and May. *E. diacanthus* spawned during April and May in Taiwan (Chen *et al.*, 1980), though it is a protracted spawner along the Western Indian Ocean region. Bouin and Siau (1983) reported that *E. aneus*, *E. guaza* and *E. alexandrinus* have the same ripening pattern of gonads in the Tunisian waters. According to Vadiya (1984), spawning season of *E. aneus* was from June to

September and that of *E. alexandrinus* was May to August. Abu –Hakima (1987) has reported that the spawning period of *E. tauvina* in Kuwait waters occurs from April to May and is associated with increasing water temperature and relatively low salinity. Tucker (1994) has reviewed spawning of serranids in captivity. Brule *et al.*,(1999, 2003) have described the reproduction in red grouper *E. mario* and the black grouper *Myctoperca bonaci* respectively, from the southern Gulf of Mexico and Bullock *et al.*, (1992, 1996) on the reproduction of jewfish *E.itajara* and the yellowedge grouper *E.flavolimbatus* from the eastern Gulf of Mexico. Chen *et al.*, (1980) estimated the fecundity of *E.diacanthus* to range from 63×10^3 to 233×10^3 According to Abu-Hakima (1987) fecundity estimates for *E.tauvina* of length 35.1 to 62.3cm ranged from 850186 to 2904921 while in *E.aneus*, the relative fecundity increases with length, whereas it decreases with increasing size in *E.alexandrinus* (Vadiya 1984). Bouian and Siau (1983) reported that equal length group of *E.aneus* (fecundity-643922) is more fecund than *E.guaza* (fecundity-606246) and *E.alexandrinus* (435202). Tessy (1994) estimated the average fecundity for *E.diacanthus* as 57458 and for *E. bleekeri* to be 737371, from Indian waters. Moe (1969) determined that the mean number of eggs of 14 gonads from *E.morio* was 1469,200; Ghorab *et al.*/ (1986) estimated the fecundity for *E.chlorostigma* from the red Sea as 315406 for 38cm long fishes and that for 52cm long fishes as 713592.

The eggs of *E.morio* were described by Moe(1969) as being less than 1mm in diameter, containing a single oil droplet and without any filaments or appendages. Ukawa *et al* .(1966) described the eggs of *E.akaara* as pelagic, spherical in shape and measuring

0.70 to 0.77mm in diameter. Colin *et al.*, (1987) have reported the egg diameter of *E.guttatus* and *E.striatus* to be 0.97 and 0.96mm respectively. Thompsun and Munro (1983) found that egg diameters of *E.guttatus* vary between 0.70 and 0.90mm. Tucker (1994), in a review of spawning by captive serranid fishes ascribes the smallest size of serranid egg to be that of *Centropristis striata* as 610µm spawned through inducement using HCG in North Carolina and the biggest size of egg to be that of *Epinephelus amblycephalus* (1000µm), spawned in Taiwan by inducement using HCG.

Ukawa *et al.*, (1966) described the early life history of *E.akaara* from the western Pacific. Presely (1970) described 16 larval specimens of *E.niveatus*, collected from the Florida straits. Hussain and Higuchi (1980) could rear the larvae of *E.tauvina* obtained by natural spawning of the fish in captivity to metamorphosis, using rotifers, artemia nauplii, copepods and minced shrimp meat as food. Glamuzina *et al.*,(1998) was able to spawn the Mediterranean dusky grouper *E.marginatus* through artificial inducement using HCG, and rear the early stages of the larvae. Lin *et al.*, (1986) by injecting hormones was able to breed the brood fishes of *E.salmonoides*, collected from the wild, and reared the larvae to fully metamorphosed fingerlings. James *et al.*, (1997) could spawn the captive reared camouflage grouper *E. polyphekadion* in the hypersaline waters of Saudi Arabia; Lim *et al.*, (1990) and Lim (1993) , reported on the breeding and larviculture of brown-marbled grouper *E. fuscoguttatus* and the greasy grouper *E.. tauvina* in Singapore. Chen *et al.*, (1977) and Chao and Lim (1991) have attempted artificial spawning and larval rearing of grouper *E.tauvina* in Singapore. In Philippines, spontaneous spawning of *E.suillus* in a tank and in floating netcage (Toledo *et al.*,1993)

and induced spawning and larval rearing of *E.salmoides* (Kungvankij *et al.*, 1986) were reported. Duray *et al.*, (1997), have attempted the larval rearing of orange spotted grouper under laboratory conditions in the Philippines.

Grouper aquaculture is in vogue in Singapore, Thailand, Malaysia, Philippines, Indonesia, Japan and Taiwan for many years (Anon, 1992). In view of the increasing demand for groupers in the export market, there is urgent need to step up production through aquaculture. Though India is the second major producer in Asia in the aquaculture sector (Asia contributes 84% in the world aquaculture production, FAO, 2002), finfish production from mariculture is almost nil. In the area of marine finfish culture, India is still in the experimental stage only. For developing economically viable and sustainable aquaculture, timely availability of pure and sufficient quantity of seeds is highly essential. This can be ensured only through supply of hatchery-produced seeds. An efficient hatchery and seed production system require a thorough knowledge about the reproductive physiology, broodstock development methods, breeding biology, seed production methods etc of the candidate species. Knowledge of the reproductive physiology and also the breeding biology of *E. tauvina* which is a highly suitable candidate species for culture along our Indian coasts is attempted in the present study.

The inshore regions all along the Indian coast are suitable for mariculture using floating net cages, though location specific work is yet to be done. The Chilka lake along the north east coast, the Pulicat lake, the Gulf of Mannar and the Palk Bay on the southeast, the Vizhinjam Bay, the backwaters along Kerala and Karnataka coasts, the

Goa coast, the Gulf of Kutch along the northwest coast, the shallow lagoons in the Andaman and Lakshadweep islands are available for pen and cage culture, but their suitability for commercial ventures need to be studied.

Chapter II.

Taxonomy and distribution of commonly occurring groupers along the South West coast of India.

2.1. Introduction

Fishes of the genus *Epinephelus* belonging to family Serranidae and coming under the sub family Epinephelinae are generally with body oblong or elongate, often stout, more or less compressed, covered with small cycloid or ctenoid scales adherent or often embedded in the skin. Lateral line complete but not very conspicuous, running parallel to the curve of back and not extending to the caudal. Head entirely scaly or nearly so. Mouth large or moderate, protractile, not very oblique, maxillary broadened distally. Pre-operculum moderately serrate, operculum with one to three spines. They are distributed in the seas in the tropic as well as temperate regions around the world. Groupers are common predatory reef fishes of worldwide tropical/warm-temperate seas; a few species are however abundant and commercially important in temperate waters. Except for breeding aggregations, most species of groupers are solitary living. All are predators on fishes and invertebrates including crabs and lobsters. Most of the serranids are either synchronous or transforming hermaphrodites that begin life as females and later become males. This family includes a large number of species ranging in size from a few

centimeters to over 2m and weight 400kg. Most of them are excellent food fishes much saught in commercial fisheries, others are of local interest to sports-fishermen and in subsistence fisheries. They are mostly taken in traps, on hooks and lines or on long lines and those inhabiting soft bottoms are caught in bottom trawls.

Phylogenetic systematics among the groupers are poorly known. Relationships among the genera have been the subject of much disagreement. According to Weber and De Beaufort (1931) Genus Epinephelus forms among one of the 5 genera of Subfamily Epinephelinae namely *Variola, Epinephelus, Plectropoma, Anyperodon* and *Cromileptus* . In the chapter on systematic catalogue in Groupers of the world, Heemstra and Randall (1993) classified the sub family Epinephelinae to comprise of 15 genera viz. *Atheloperca, Alphestes, Anyperodon, Cephalopholis, Cromileptis, Dermatolepis, Epinephelus, Gonioplectrus, Gracilia, Myctoperca , Paranthias, Plectropomus, Saloptia, Triso* and *Variola*; the genus *Epinephelus* comprises of maximum number of 98 species of serranid fishes which are the highest priced fishes. Heemstra and Randall (1993) classified the serranid sub family Epinephelinae to be represented by 15 genera and 159 species. Smith (1971) demoted the genus *Promicrops* comprising *E. itajara* and *E. lanceolatus* to a subgenus of *Epinephelus* and stated that these two species are highly specialized and distinctive although their alliance with other species of *Epinephelus* is clear. Smith (1971) included the genera *Alphestes, Cephalopholis* and *Dermatolepis* in *Epinephelus* because it appears that *Epinephelus* is more closely related to the genus *Myctoperca.* The species of both genera have the base of the soft- rayed part of dorsal fin

shorter than or equal to the base of the spinous part; they have only bisegmental pterigiophores supporting dorsal and anal fins.

Groupers are not long-distance or fast swimmers, but rather choose to lie, wait, and ambush their prey with a quick flash of their powerful jaws. In general groupers are classified as "unspecialized carnivores". Most groupers do not have teeth in their jaws to rip apart their prey, but instead are equipped with powerful mouths and gills that create a sucking system that pulls prey into their mouth from a long distance. In addition, they have teeth plates inside their pharynx to crush their prey and to prevent them from escaping after being swallowed.

Order PERCOMORPHI

Sub order Percoidea

Division Perciformes

Family Serranidae.

Subfamily Epinephelinae.

Genus Epinephelus

Genus Epinephelus

Key to Genus Epinephelus.

1. Dorsal fin spines IXto XI; lower edge of preopercles smooth ---------- **2**

2. Caudal fin rounded, truncate or concave----------**3**

3. Palatines with teeth; body compressed in some species, but its width contained less than 3 times in head length ----------**4**

4. Dorsal profile of head straight, convex or slightly concave; rear nostrils round or oblong ----------**5**

5. No large anterorostral spine on corner of preopercle (but a few enlarged, ventrally directed serrae present there); snout longer than orbit diameter ----------**6**

6. Pectoral fins symmetric or nearly so, the middle rays longest; dorsal fin with IX to XI spines and 12 to 21 rays; caudal fin rounded, truncate or emarginated ----------**7**

7. Dorsal fin spines X or XI ----------**8**

8. Body elongate, robust, robust, to deep and compressed, the depth 2.3 to 4.1 times in standard length, usually less than head length; dorsal fin with IX to XI spines and 12 to 19 rays ----------**9**

9. Anal fin rays 10 to 13; body depth at dorsal fin origin not more than depth at anus; caudal fin usually rounded (truncate in some species, but rarely emarginate or concave).

Grouper species are identifiable by their colour pattern and/or a suite of morphological characters including body shape, configuration and size of the fins, the shape and relative size of the head and body, the number of fin rays, scales and gill rakers. Except in large adults of some species the colour pattern of most groupers is usually distinctive enough to identify the particular species. Juveniles of some species look completely different from adults of the same species. In some species with dark spots, the spots become smaller and more numerous with growth. Colour pattern can

often be altered in a few seconds, depending on the mood of the fish. Many groupers have a fright or stress pattern of white blotches or bars.

Groupers have robust somewhat compressed oblong-oval to rather elongate body; mouth with small, slender, depressible teeth on jaws, vomer and palatines, distinct canine teeth present at front of mouth in some species; no molars or incisiform teeth; maxilla exposed when mouth is closed, with or without supramaxilla. A single dorsal fin with VII to XI spines and 10 to 21 rays; anal fin III spines and 7 to 13 rays; caudal fin rounded or truncate in most species, emarginated to lunate in a few with 13 to 15 branched rays; pelvic fins with 1 spine and 5 branched rays, no scaly process at base of pelvic fins; pectoral fins broadly rounded, the base scaly. Edge of preopercle serrate; opercle with 2 or 3 flat points or spines ; most species have three distinct spines; gill membranes separate, joined to isthmus with 7 branchiostegal rays. Anterior and posterior nostrils close together. Scales small, adherent, ctenoid or cycloid. Lateral line single. Colour patterns are generally the most useful field characters as the morphometric and meristic characters often overlap.

Commonly known as groupers, rock cods, hinds, coral groupers etc. these are of considerable economic value. Groupers are bottom associated fishes found in the coastal fisheries of tropical and subtropical waters of all oceans. Most species occur on coral reefs, but some live in estuaries or on rocky reefs. Though associated with rocky bottoms, juveniles are found in seagrass beds and adults of a few species prefer sandy or silty areas. Some species occur in depths of 10 to 200m, occasionally to 500m but majority

inhabit depths less than 100m and juveniles are often found in tide pools. Two large species, *E . itajara* and *E. lanceolatus* grow well over 2 meters in length and weight of over 400kg. Except for spawning aggregations most species are solitary fishes, tagging studies show that groupers are resident on a particular reef for long periods (Heemstra and Randall, 1993). The site specificity and the relatively slow growth rate make groupers particularly vulnerable to over-fishing. Some groupers use localized spawning grounds to which they migrate from distant places and fishermen who catch large numbers in the brief spawning period often exploit from these sites. This removal of considerable number of reproductively active fish from the population may be detrimental to sustained yields from the fishery.

The reproductive biology of a few species has been studied; they appear to be protogynous hermaphrodites that start their sexual life as females and later transform into males; a few have separate sexes. Most of the species are taken in traps, on hooks and lines, on longlines and those inhabiting the soft sandy or muddy bottoms are caught by trawls. The taxonomic status of the commonly occurring commercially important species were studied.

2.2. Materials and methods

Samples for this study were collected from the commercial landings at the Cochin Fisheries Harbour, brought by the Kolachal fishermen who carry out hook and line fishing operations for kalava from the perch grounds off Cochin and Ponnani. The sampling was carried out during the period October 1998 to March 2000. All the six

species selected were commercially the most important ones contributing to the fishery. Fishes were taken in fresh condition to the laboratory, all meristic and morphometric measurements were observed and the weights were taken to the nearest milligram (Tables 1 &2). Each measurement was taken and recorded to the nearest millimeter. All diagnostic features, fin counts, colour patterns and special characters were observed in fresh condition as groupers being inhabitants of rocky and coralline habitat exhibit vivid colour pattern. Colour descriptions of species are based on specimens in fresh condition. Colour photographs also were taken in fresh condition using a digital camera. The following measurements and counts are used in the present study.

1. Total length – Distance from tip of snout to tip of upper lobe of caudal fin.

2. Standard length – Distance from tip of snout to mid base of caudal fin.

3. Head length – Distance from tip of snout to hind edge of the opercular flap.

4. Snout length – Distance from tip of snout to anterior edge of orbit.

5. Eye diameter – Horizontal orbit distance to edge of bony borders.

6. Pre-dorsal length – Distance from tip of snout to anterior margin of the insertion of dorsal fin.

7. Depth of body - Origin of first dorsal spine to insertion of pelvic spine.

8. Inter orbital length – Least distance between dorsal bony edges of the eyes.

9. Pre-pectoral length - Distance from tip of snout to anterior margin of the insertion of pectoral fin.

10. Pre- pelvic length - Distance from tip of snout to anterior margin of the insertion of pelvic fin.

11. Gill rakers - All elements counted.

12. Lateral line scales – Pored scales from post temporal bone to base of hypurals plate

13. Dorsal fin spines – Number of spines on the dorsalfin.

14. Anal fin spines – Number of spines on the anal fin.

15. Number of colour bands /spots on the body.

16. Depth of caudal peduncle – Dorso-ventral distance of caudal peduncle at the base of caudal fin.

The relationship between certain body lengths and between certain dimensions in the head were calculated after ascertaining the type of relationship through a scatter diagram following the least square method. (Snedecor and Cochran 1967). The results are presented in figures and the calculated values of slope and elevation, alongwith the value of coefficient of determination (R^2) are shown in the **(figures 2-7)** for each species. Since body proportions are known to vary with growth, this study is of importance. Understanding such variations in growth will help in understanding the intraspecific variations in each species.

Under each description, the number of specimens examined is indicated alongwith length range and under the descriptions of each species, the recorded distribution of the species in Indian waters is given.

2.3. Description of species

Epinephelus tauvina (Forsskal, 1775), Greasy grouper.

(Plate I a)

Synonyms :

Perca tauvina Forsskal, 1775,

Serranus salmoides Day, Fishes of India, 1888.

Serranus pantherinus Day, Fauna of India, Fishes 1 . 1889;

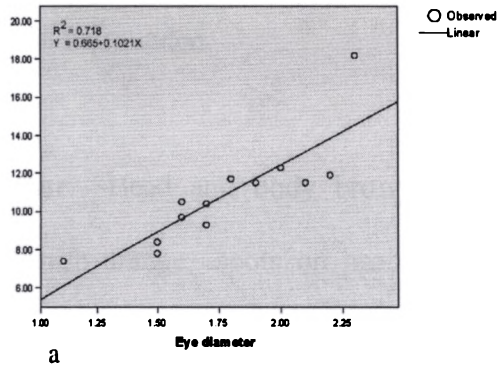
Serranus salmoides Hora, Mem. Asit. Soc. Bengal VI. 1924, p 486

Serranus tauvina Fowler & Benn ,Proc.U.S.Nat. Mus. LXXI, 1927, p 6.

Number of specimens examined 20nos , in the size range 120mm – 560mm, collected from Off Cochin

Diagnostic features: Body elongate, the depth contained 3.0 to 3.6 times in standard length . Head large, its length contained 2.1 to 2.4 times in standard length; snout length contained 2.0 to 2.4 times in upper-jaw length; inter-orbital area narrow, flat or slightly concave; pre-opercle broadly rounded (not angular), serrae at the corner of pre-opercle slightly enlarged; upper edge of operculum almost straight; nostrils sub equal, maxilla reaching well past eye, midlateral part of lower jaw with 2 to 4 rows of teeth. Gill rakers 8 to 10 on upper limb, 17 or 20 on lower limb. D XI, 15-16 rays, third to fifth spine the longest, interspinous dorsal fin membranes incised; anal fin with III spines and 8 rays,

Fig. 2



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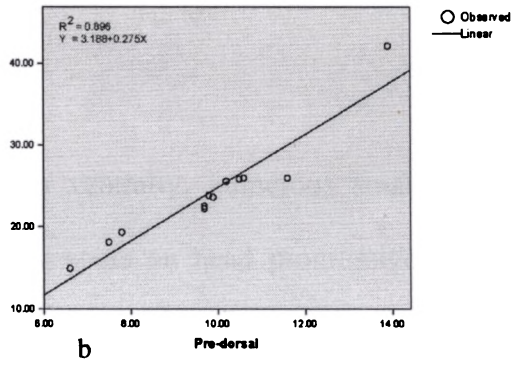

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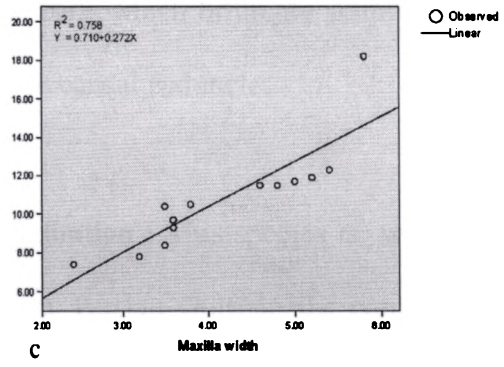

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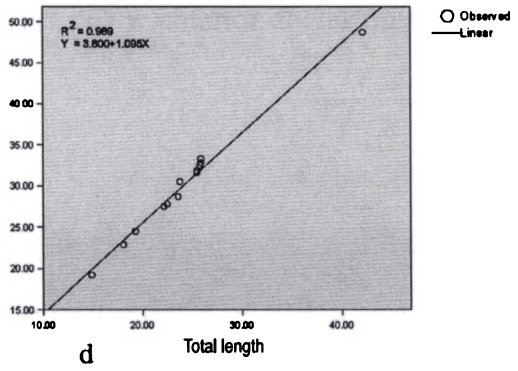

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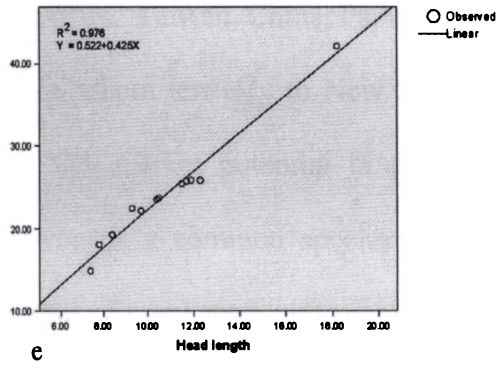

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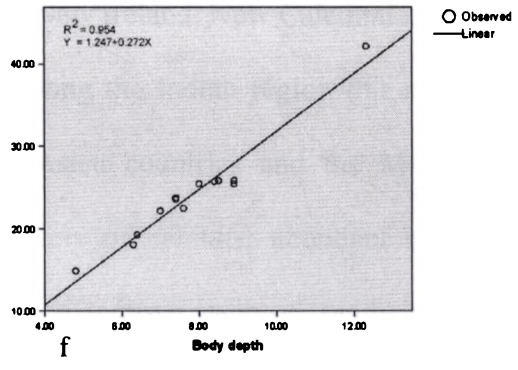

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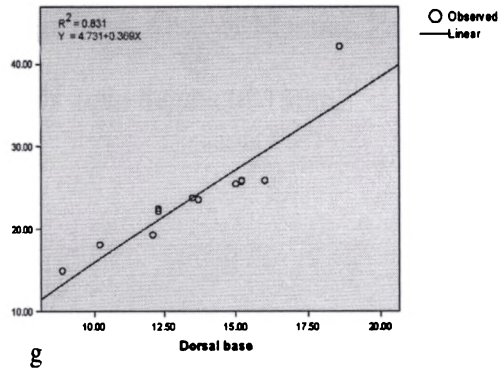


g

E-tauvina

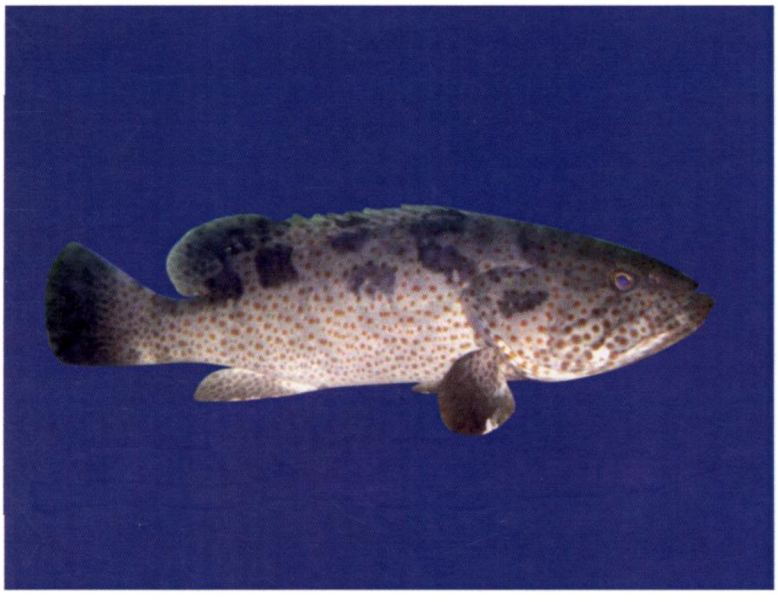
a : Eye diameter on head length
b : Pre-dorsal length on Standard length
c : Maxilla width on head length
d : Total length on Standard length
e : Head length on standard length
f : Body depth on Standard length
g : Dorsal base on Standard length

the third spine longer than second; lateral line scales 65 to 69; pectoral fin rays 18 to 19, caudal fin rounded.

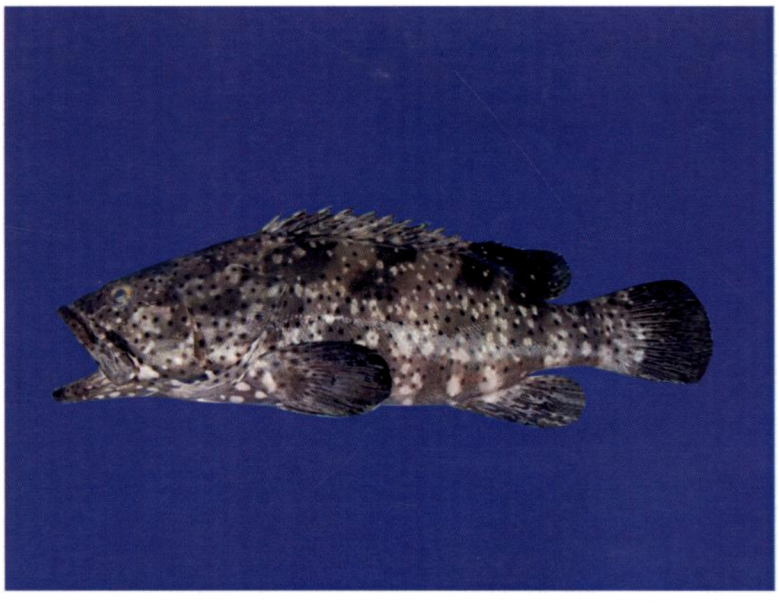
Colour: Head and body brownish to greenish ventrally; numerous small roundish brownish orange spots on head, body and fins; spots on head progressively smaller anteriorly, fins also covered with dark spots; body with five faint, irregular, oblique, dark bars which bifurcate ventrally; first dark bar below anterior dorsal fin spines, last bar on caudal peduncle.

Distribution : This species is widely distributed in the Indo-Pacific and the Red Sea region, Persian Gulf; in the continental and insular localities of Sudan, Saudi Arabia, Ethiopia, Kenya, Tanzania, Oman, Madagascar, Comoros, India, Indonesia, Singapore, Philippines, Taiwan, China, Papua New Guinea, New Ireland, New Caledonia; Australia, from Northern territory to New South Wales. Along the Indian region this species has very high market potential in the south East Asian countries and the Middle East. *E.tauvina* is a common species found in a variety of habitats; abundant in the vast stretches of coral reefs and rocky areas estuaries, mangrove swamps, sandy and muddy bottoms; distributed along the Gulf of Kutch, Malwan (Maharashtra), the Kerala coast, Wadge Bank, Gulf of Mannar, Palk Bay and the seas around Andaman and Lakshadweep islands upto depths of 150m.

PLATE I



a. Photograph of the Greasy grouper *Epinephelus tauvina*



b. Photograph of the Malabar grouper *E.malabaricus*

Epinephelus malabaricus (Bloch and Schneider,1801)

Malabar grouper (Plate I b)

Synonyms:

Holocentrus malabaricus Bloch and Schneider, 1801:

Holocentrus salmoides Lacepede, 1802:389; 1801;pl.34,

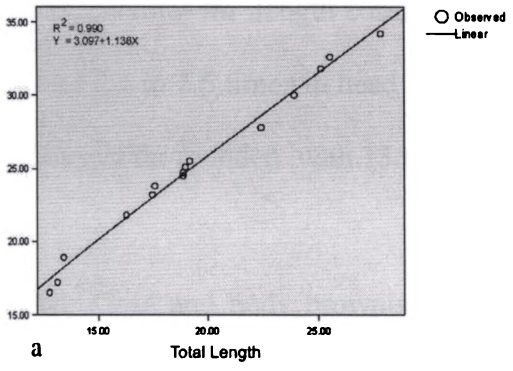
Serranus salmonoides Valenciennes in Cuv. And Val., 1829:343(emendation and redescription of *Holocentrus salmoides* L acepede, 1802).

Serranus crapao Cuvier in Cuv. And Val., 1829; 494

Number of specimens examined 20nos , in the size range 120mm – 600mm, collected from Off Cochin.

Diagnostic features: Body elongate, the depth contained 3.0 to 3.7 times in standard length; body width 1.4 to 1.9 times in depth. Head length contained 2.3 to 2.6 times in standard length; snout length contained 1.7 to 2.0 times in upper jaw length; interorbital width contained 4.5 to 6.5 times in head length and 2.1 to 3.0 times in upper jaw length; inter orbital area flat or slightly convex, preopercle subangular, with enlarged serrae at the angle; upper edge of operculum almost straight; nostrils subequal, large adults have posterior nostrils slightly larger. Maxilla extending past vertical at rear edge of orbit, width 4.5 to 6.5% of standard length; upper jaw length 17 to 22% of standard length midlateral part of lower jaw with 2 to 5 rows of teeth. Gill rakers 8 to 11 on the upper limb, 14 to 18 on the lower limb. Dorsal fin with XI spines and 14 to 16 rays, the third to fifth spines usually slightly longer than posterior spines, their length contained 3.1 to 4.0 times in head length and distinctly shorter than longest rays, the inter spinous membranes incised; anal fin III spines and 8 rays, the third spine usually longest ; pectoral fin rays

Fig. 3



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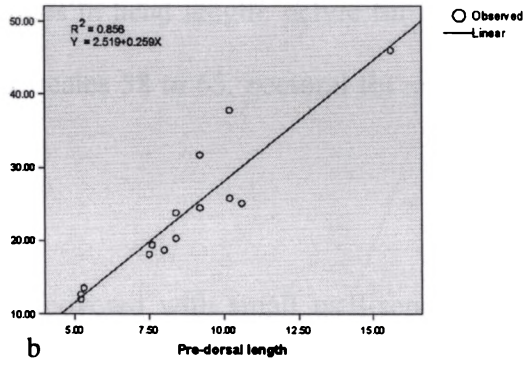

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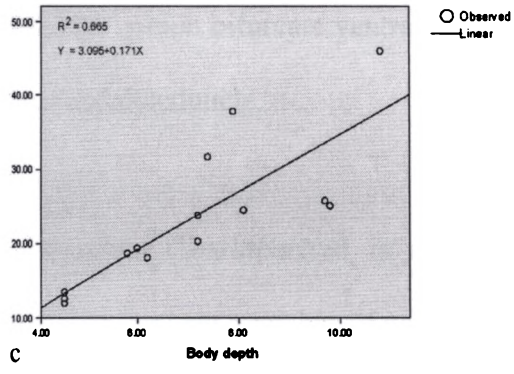

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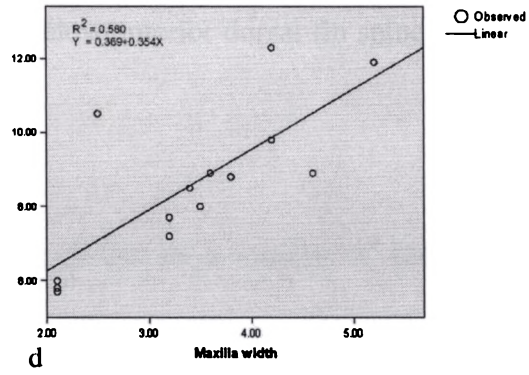

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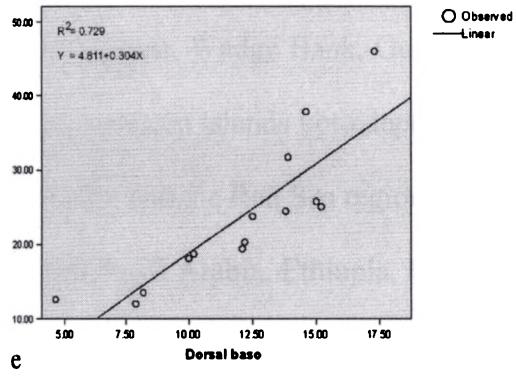

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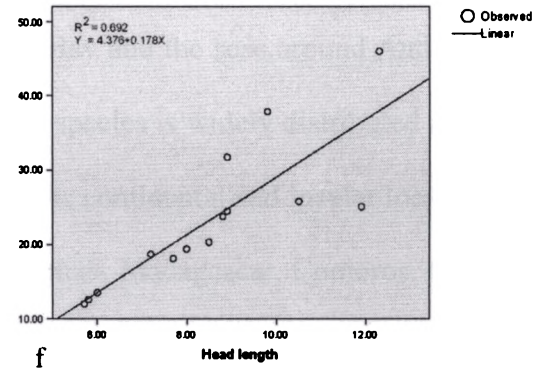

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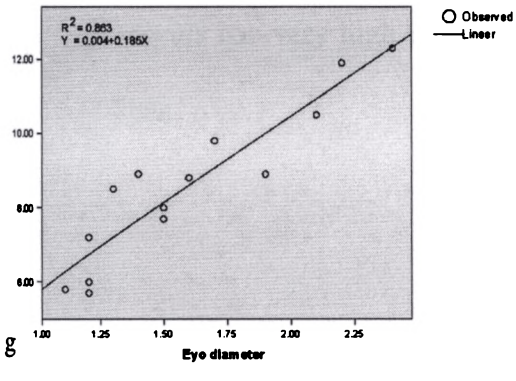


g

E-malabaricus

a : Total length on Standard length
b : Pre-dorsal length on Standard length
c : Body depth on Standard length
d : Maxilla width on head length
e : Dorsal base on Standard length
f : Head length on standard length
g : Eye diameter on head length

18 to 20; pectoral fin length contained 1.7 to 2.2 times in head length, pelvic fin length contained 2.1 to 2.6 times in head length; lateral line scales 58 to 63, pectoral fin rays 18 to 20, caudal fin rounded with 13 to 15 branched rays.

Colour: Head and body brownish to tan ventrally; covered with small well-separated blackish brown spots on head. body and fins; body with five faint, irregular, oblique, dark brown bars which bifurcate ventrally; first dark bar below anterior dorsal fin spines, last bar on caudal peduncle.

Distribution. *E.malabaricus* is a common species found in a variety of habitats; abundant in the vast stretches of coral reefs and rocky areas estuaries, mangrove swamps, sandy and muddy bottoms; distributed along the Gulf of Kutch, Malwan (Maharashtra), the Kerala coast, Wadge Bank, Gulf of Mannar, Palk Bay and the seas around Andaman and Lakshadweep islands upto depths of 150m. This species is widely distributed in the Indo-Pacific and the Red Sea region, Persian Gulf; in the continental and insular localities of Sudan, Saudi Arabia, Ethiopia, Kenya, Tanzania, Oman, Madagascar, Comoros, India, Indonesia, Singapore, Philippines, Taiwan, China, Papua New Guinea, New Ireland, New Caledonia; Australia, from Northern territory to New South Wales. Along the Indian region this species has very high market potential in the Southeast Asian countries and the Middle East.

Epinephelus diacanthus (Valenciennes, 1828).

Spinycheek grouper (Plate II a)

Synonyms:

Serranus sexfasciatus Day Fishes of Malabar, 1865, p.2.

Epinephelus diacanthus Boulenger , Cat.Brit. Mus. 2nd ed. I. 1895, p.209.

Epinephelus diacanthus M. Weber, Siboga Exp. Fische 1913, p.202.

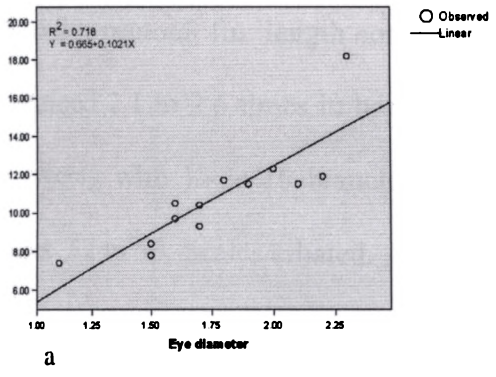
Serranus diacanthus Hora, Mem. Asiat. Soc. Bengal VI. 1924, p 486.

Epinephelus diacanthus Bernard, Ann.S. Afric. Mus. XXI. 1925-1927, p.278.

Number of specimens examined 20nos , in the size range 100mm – 450mm, collected from Off Cochin.

Diagnostic features: Body elongate, the depth 2.8 to 3.5 times in standard length; interorbital flat or slightly convex . Lower jaw projecting, mouth oblique, maxillary extending to below posterior border of eye. Teeth in narrow bands; outer row of fixed teeth and canines strong, pre-operculum finely serrated behind, its border forming at right angle; with 1 to 3 enlarged serrae at the angle; operculuar flap pointed; nostrils subequal, the margin usually with a large , bilobed flap of skin . Head covered with cycloid scales, maxillary naked or partly scaly. Gill rakers shorter than gill fringes, 14-15 on thelower part of the anterior arch. Dorsal fin originating above base of pectorals, XI spines and 15 to 17 rays; 3rd and 4th spines longest ; the inter spinous membranes incised; anal fin III

Fig. 4



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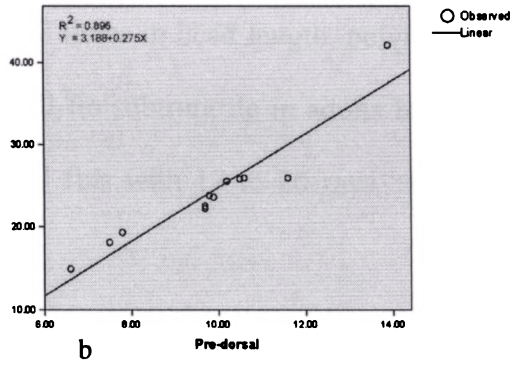

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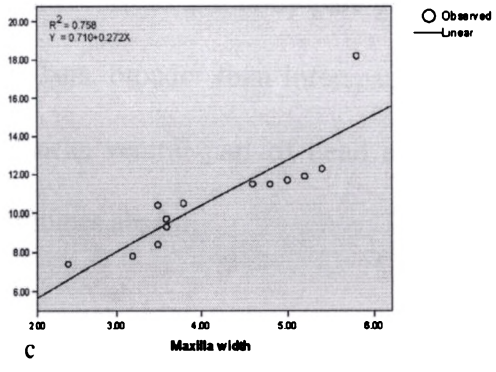

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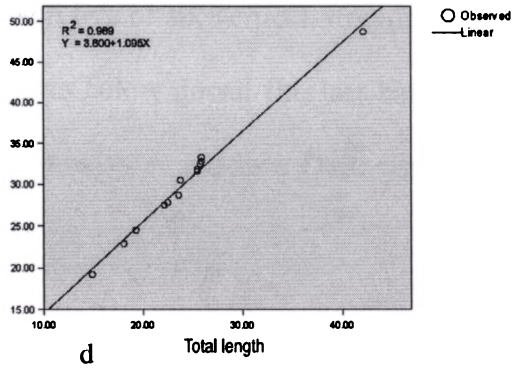

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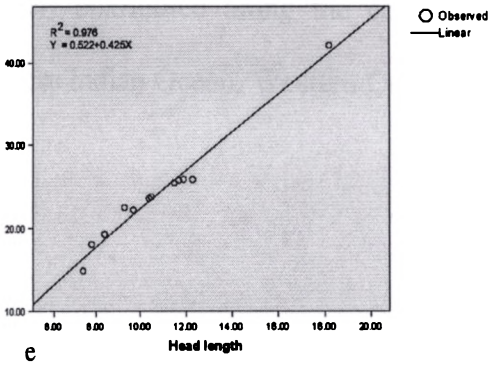

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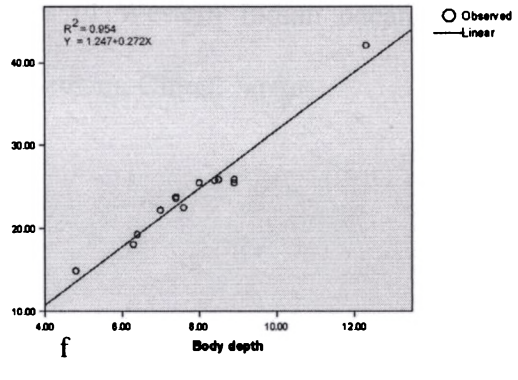

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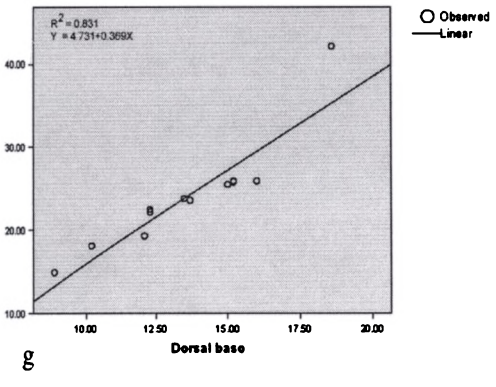


g

E-diacanthus

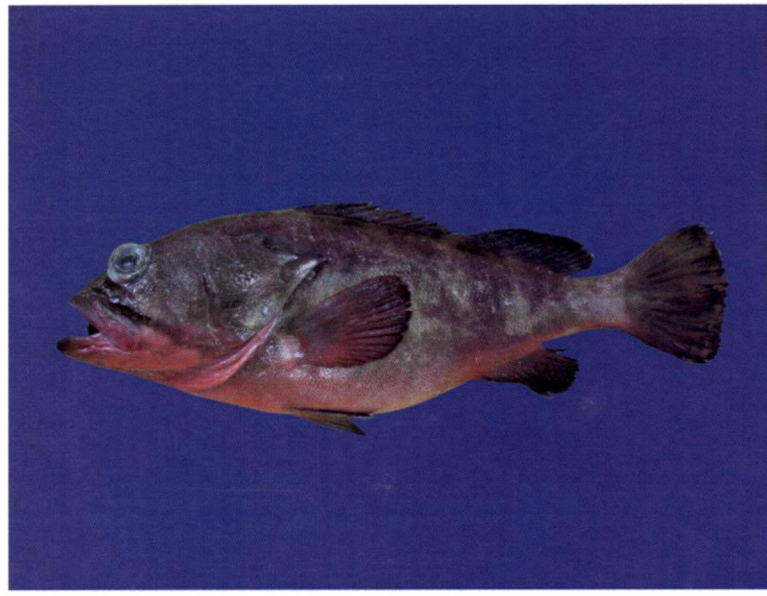
a : Eye diameter on head length
b : Pre-dorsal length on Standard length
c : Maxilla width on head length
d : Total length on Standard length
e : Head length on standard length
f : Body depth on Standard length
g : Dorsal base on Standard length

spines and 8 rays, the second stronger than and as long as third spine; pectoral fin rays 18 to 20; pectoral fin length contained 1.7 to 2.2 times in head length, pelvic fin length contained 2.1 to 2.6 times in head length; caudal fin subtruncate in adults but rounded in young with 13 to 15 branched rays, pectoral fins with 17 to 20 rays. Lateral line scales 52 to 56; Scales ciliated.

Colour: Head and body pale grayish brown with five or six vertical, irregular, oblique, dark bars, broader than interspaces; four dark bars below dorsal fin, last bar on caudal peduncle; ventral part of head and body often pinkish or reddish. Dark bars on body sometimes absent.

Distribution: A fairly deep-water species, known to occur from 10 to 200m depth. Widely distributed along the continental shelves of Western Indian ocean, Red sea, Eastern Indian Ocean, Western Central Pacific, Vietnam, China, Japan.

PLATE II



a. Photograph of Spiny cheek grouper *Epinephelus diacanthus*

b. Photograph of Brown spotted grouper *E.chlorostigma*

Epinephelus chlorostigma (Valenciennes, 1828).

Brownspotted grouper (Plate II b)

Synonyms:

Serranus areolatus Cuvier & Valenciennes, Hist. Nat. Poissons II, 1828,p350.

Serranus areolatus Day, Fishes of India 1878-1888, p.12.

Epinephelus chlorostigma Boulenger, Cat. Brit. Mus. 2nd ed. 1895, p.203.

Epinephelus chlorostigma Jordan, Tanaka & Sinder, Jour. Coll. Science Univ. Tokyo XXXIII. Art.1, 1913, p.153.

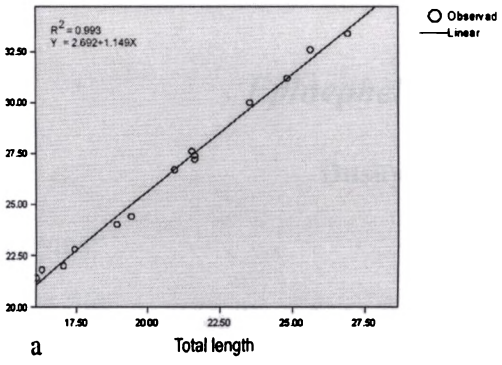
Number of specimens examined 20nos , in the size range 100mm – 350mm, collected from Off Cochin.

Diagnostic features: Body depth contained 2.8 to 3.3 times in standard length; head length contained 2.4 to 2.7 times in standard length, eye diameter 5.0 to 7.3 times in head length, interorbital slightly convex; preopercle angular, with 4 to 7 enlarged serrae at angle; upper edge of operculum straight; maxilla reaches about to vertical at rear edge of eye; maxilla scaly. Midlateral part of lower jaw with 2 to 4 rows of teeth, the inner ones about twice the size of outer teeth. Gill rakers longer than gill filaments. Dorsal fin with XI spines and 16 to 18 soft rays, third or fourth spine longest, its length contained 2.4 to 3.2 times in head length, dorsal fin not much incised between spines; anal fin rounded or angular, with III spines and 8 rays, the third spine longer than the second; pectoral fin rays17 to 19, slightly longer than pelvic fins; caudal fin truncate or slightly emarginated. Pored lateral line scales 49 to 53.

Colour: Head, body and fins with small, close- set, small, irregular brown spots; a narrow white edge visible along the rear margin of caudal fin, spots on pectoral fins confined to rays, ventral margin of anal fin dusky.

Distribution: A fairly deep-water species, known to occur from 10 to 200m depth. Found in coral reefs and also on muddy bottoms. Widely distributed along the continental shelves of Western Indian ocean, Red sea, and the Persian Gulf, east coast of Africa to the western Pacific; but not recorded from Comoros, continental shelf between Oman and Cambodia, Indonesia, Philippines, Taiwan and Australia.

Fig. 5



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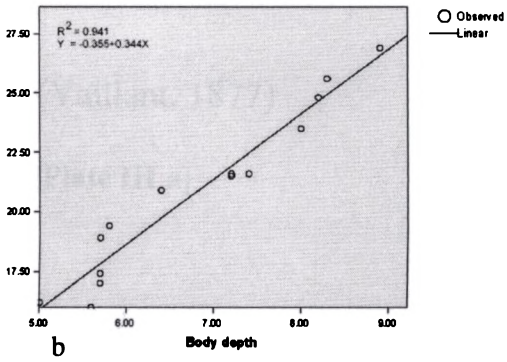

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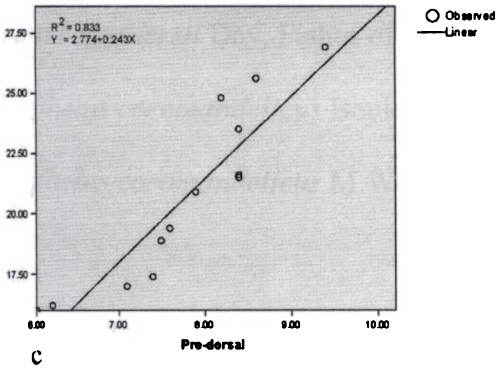

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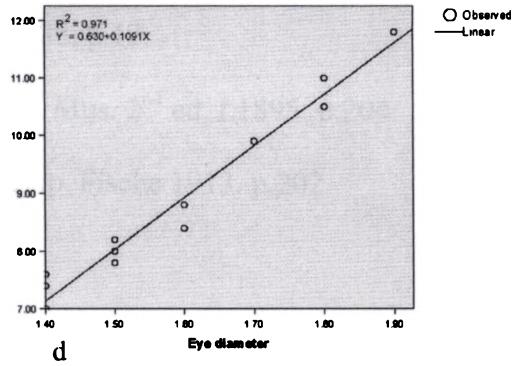

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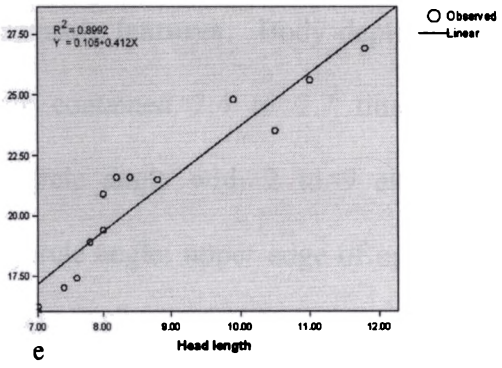

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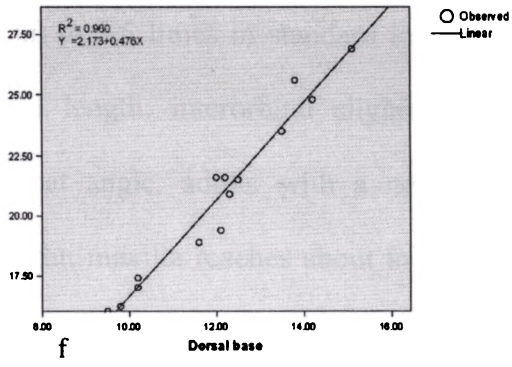

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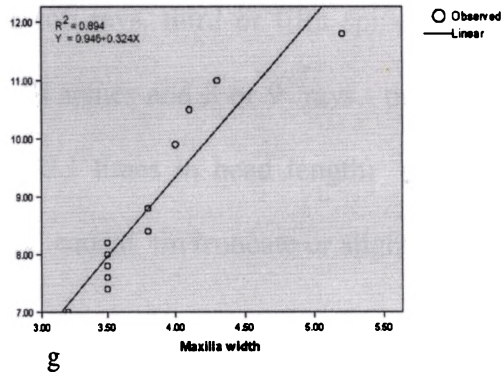


g

E-chlorostigma

a : Total length on Standard length
b : Body depth on Standard length
c : Pre-dorsal length on Standard length
d : Eye diameter on head length
e : Head length on standard length
f : Dorsal base on Standard length
g : Maxilla width on head length

Epinephelus bleekeri (Vaillant, 1877)

Duskytail grouper (Plate III a)

Synonyms:

Serranus Bleekeri Vaillant in Vaillant and Bocourt, 1877: 47 and 69 (based on *Serranus variolosus* [non Valenciennes]: Bleeker, 1849).

Serranus wandersii Day, Fishes of India, 1878-1888, p.12.

Epinephelus coromandelicus Boulenger, Cat. Brit. Mus. 2nd ed. I.1895, p.204

Epinephelus coromandelicus M.Weber, Siboga Exp. Fische 1913, p.202

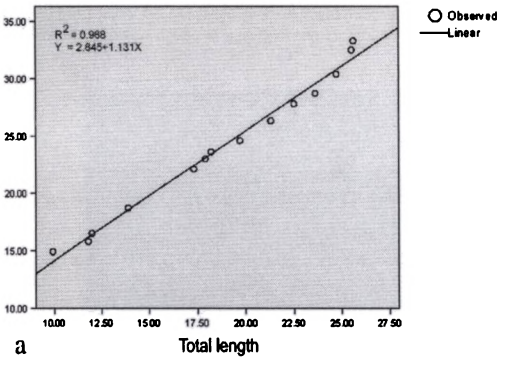
Number of specimens examined 20nos, in the size range 100mm – 450mm, collected from Off Cochin.

Diagnostic features: Body depth contained 3.0 to 3.5 times in standard length; head length contained 2.4 to 2.7 times in standard length, interorbital slightly convex; preopercle angle with 2 to 9 enlarged serrae at angle, adults with a notch above preopercle angle; upper edge of operculum straight; maxilla reaches about to vertical at rear edge of eye; maxilla scaly. Midlateral part of lower jaw with 2 rows of subequal teeth, the inner ones about twice the size of outer teeth. Dorsal fin with XI spines and 16 to 18 soft rays, third or fifth spines longest, interspinous membrane incised ; anal fin with III spines and 8 or 9 rays, pectoral fin rays17 to 19, pectoral fin length contained 1.6 to 2.1 times in head length; pelvic fin length contained 1.9 to 2.5 times in head length; caudal fin truncate or slightly convex. Pored lateral line scales 50 to 54.

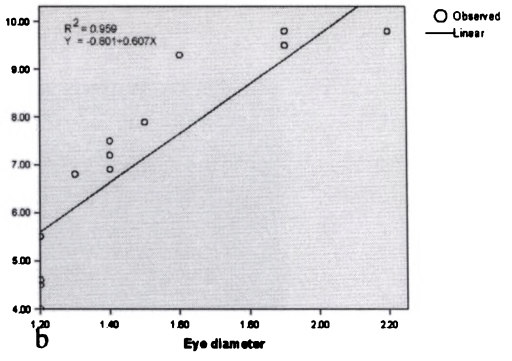
Colour: Head and body brownish, reddish brown, covered with numerous reddish orange, gold or yellow spots; lower two thirds of caudal fin dusky, spots on body of some fish with a faint dark margin; pectoral and pelvic fins and distal part of anal dusky, dark streak along maxillary groove. Juveniles with 7 faint daint dark bars dorsally on body; no dark spots on head or fins, pectoral fins pale.

Distribution: Found in coral reefs and also shallow rocky banks, widely distributed along the continental shelves of Indo-West Pacific, Indian ocean, Red sea, and the Persian Gulf to Taiwan, Indonesia, Philippines. China, Hong Kong Taiwan and Australia.

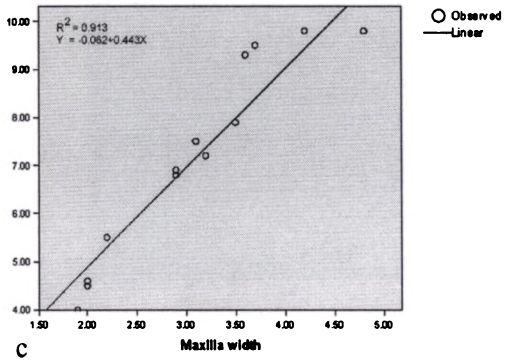
Fig. 6



a



b



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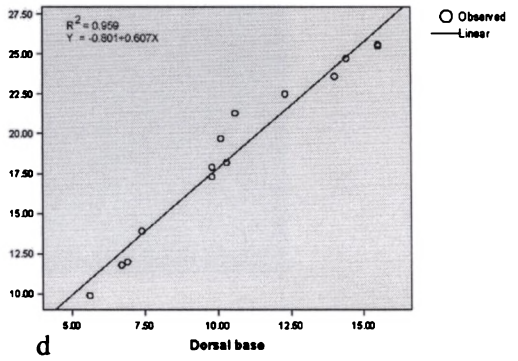

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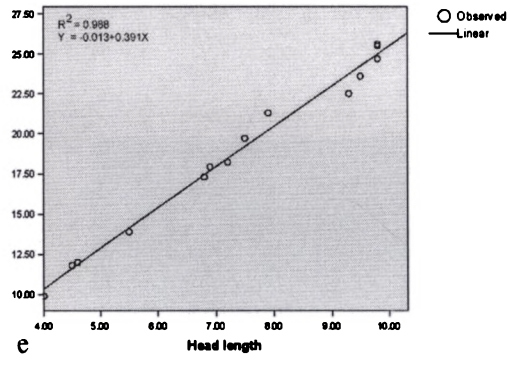
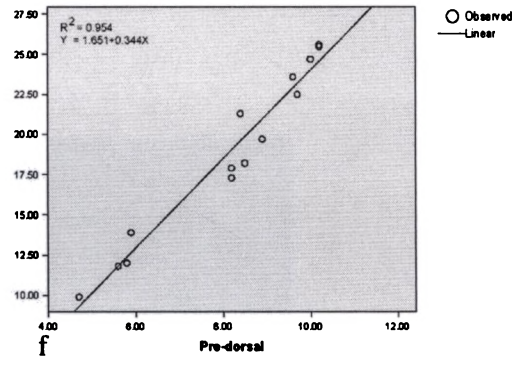


e



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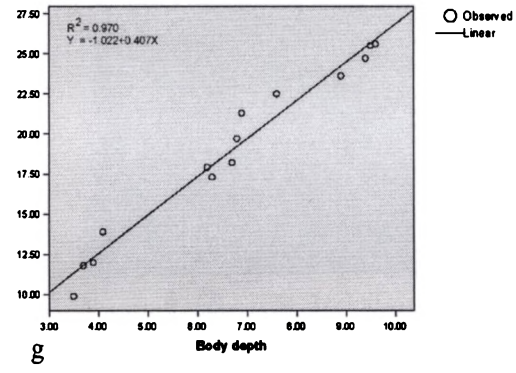


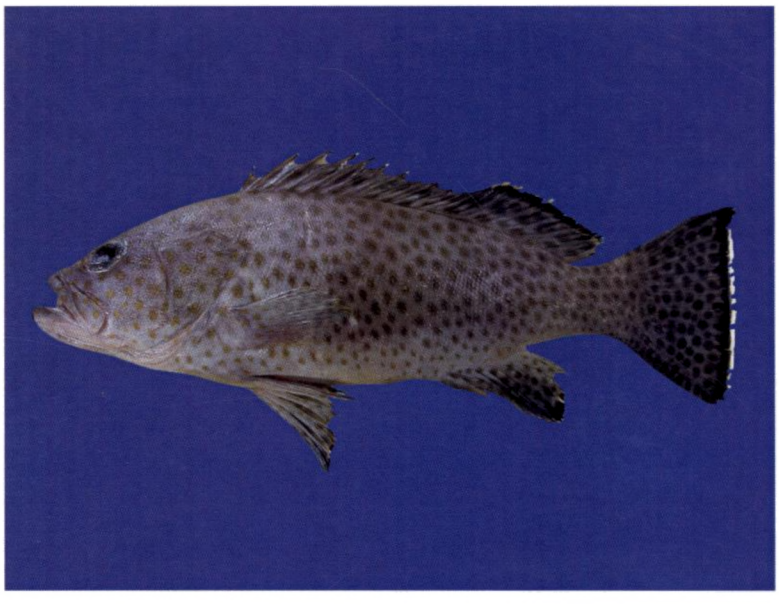
g

E-bleekeri

a : Total length on Standard length
b : Eye diameter on head length
c : Maxilla width on head length
d : Dorsal base on Standard length
e : Head length on standard length
f : Pre-dorsal length on Standard length
g : Body depth on Standard length

PLATE III

a. Photograph of the Dusky tail grouper *Epinephelus bleekeri*



b. Photograph of the Areolate grouper *E.areolatus*

Epinephelus areolatus (Forsskal, 1775)

Areolate grouper (Plate III b)

Synonyms:

Perca areolata Forskal, Descript. Animal. 1775, p.42.

Serranus celebicus Gunther, Cat. Brit. Mus. 1.1859 , p.139

Serranus glaucus Day, Proc. Zool. Soc. London 1870, p.678

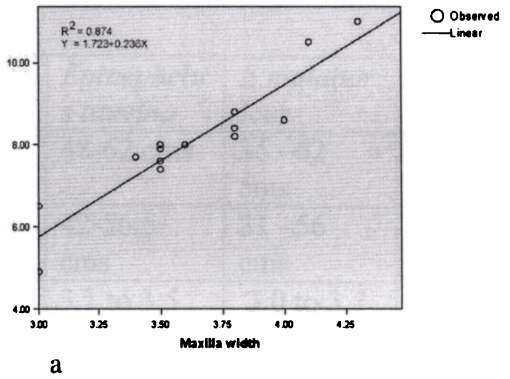
Serranus angularis Day, Fishes of India, 1878-1888, p.22

Epinephelus areolatus Boulenger, Cat. Brit. Mus. 2nd ed. 1895, p.202.

Number of specimens examined 20nos . in the size range 100mm – 400mm, collected from Off Cochin.

Diagnostic features: Body depth contained 2.8 to 3.3 times in standard length; head length contained 2.4 to 2.8 times in standard length, interorbital area convex ; preopercle angular, with 2 to 7 enlarged serrae at angle; upper edge of operculum straight or slightly convex; nostrils subequal , maxilla reaches to below rear half of eye, maxilla, lower jaw and gular area scaly. Midlateral part of lower jaw with 2 rows of teeth. Gill rakers8 to 10 on upper limb, 14 to 16 on lower limb. Dorsal fin with XI spines and 15 to 17 soft rays, third or fourth spine longest and subequal to longest ray, the interspinous membranes moderately incised ; anal fin rounded or angular, with III spines and 8 rays, pectoral fin rays17 to 19, longer than pelvic fins; caudal fin margin truncate to slightly emarginated. Pored lateral line scales 50 to 52.

Fig. 7



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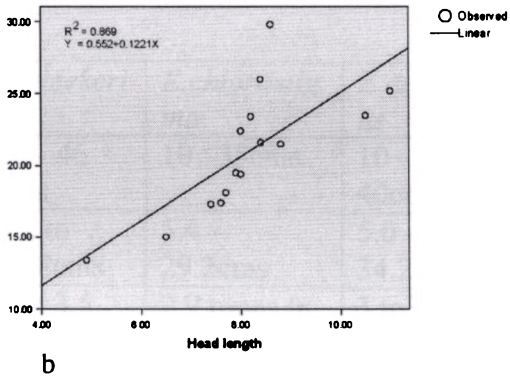
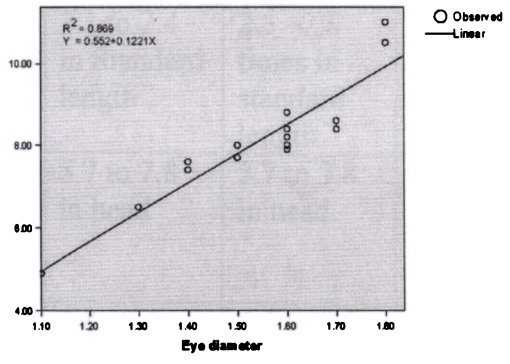
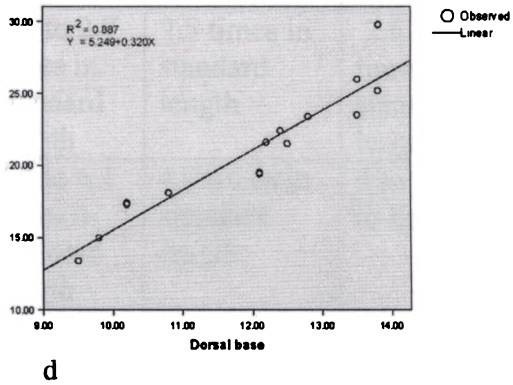


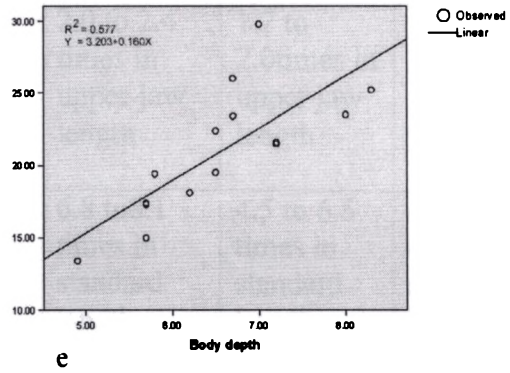
b



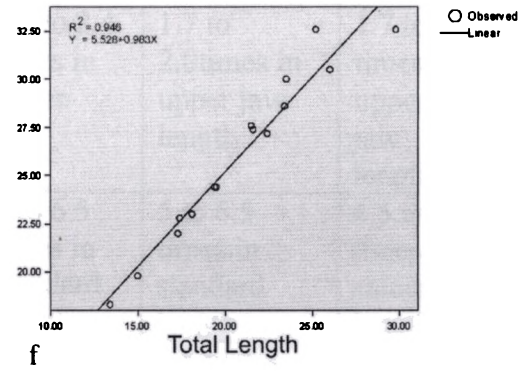
c



d



e



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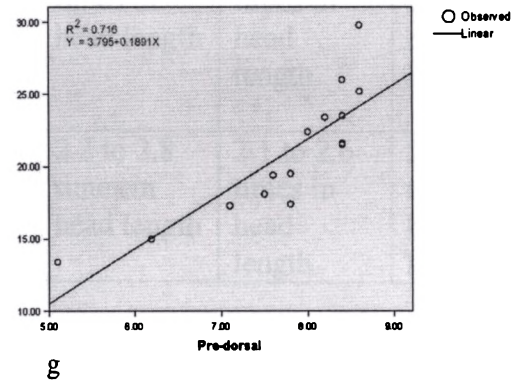


g

E-aerolatus

a : Maxilla width on head length
b : Head length on standard length
c : Eye diameter on head length
d : Dorsal base on Standard length
e : Body depth on Standard length
f : Total length on Standard length
g : Pre-dorsal length on Standard length

Table 1. ***Morphometric characters of the commercially important species of groupers.***

Characters	*Epinephelus tauvina*	*E.malabaricus*	*E.diacanthus*	*E.bleekeri*	*E.chlorostigma*	*E.areolatus*
Total length.	48-62 cms	36 –62 cms	18 – 56 cms	12 - 46 cms	10 –35cms	10 – 40cms
Standard length	42-56.5 cms	31 –56 cms	13 –50 cms	7.2 to 40.8cms	5.4 – 29.2cms	5.0 – 34.2cms
Depth of body	3.1 to 3.5 times in Standard length	3.0 to 3.7 times in standard length	2.8 to 3.5 times in standard length	3 to 3.5 times in standard length	2.9 times in standard length	3 to3.4 times in standard length
Head length	2.1 to 2.4 in Standard length	2.3 –2.6 times in standard length	2.2 to 2.4 times in standard length	2.5 to 2.7 times in standard length	2.5 times in standard length	2.6 times in standard length
Eye diameter	5.7 to 7.8 in head	5.7 to 7.8 in head	5.0 to 5.5 in head	4.4 to 6.1 times in standard length	4.9 times in standard length	5 to 5.4 in head
Int.Orb.width	6.8 –8.1 times in head length	4.5 to 6.5 times in head length	4.5 5.5 in head length	4.2 to 5.8 times in head length	Two thirds of eye diameter	4.5 to 5.0 in head length
Snout length	2.0 to 2.4 times in upper-jaw length	1.7 to 2.0times in upper jaw length	1.7 to 2.0times in upper jaw length	2.0 to 2.4 times in upper-jaw.	1.7 to 2.0times in upper jaw length	1.7 to 2.0 times in upper jaw length
Maxilla width	6.8 to8.1 times in standard length	4.5 to 6.5 times in standard length	4.5 to 6.5 times in standard length	5 to 6.5 times in standard length	5 to 6.5 times in standard length	4.5 to 6.5 times in standard length
Pectoral length	1.7 to2.4 times in head length	1.7 to 2.2 times in head length	1.7 to 2.1 times in head length	1.6 to 2.1 times in head length	1.8 to 2.3 times in head length	1.5 to 1.8 times in head length
Pelvic fin length	2.2 to 2.8 times in head length	2.1 to 2.6 times in head length	2.0 to 2.6 times in head length	1.9 to 2.5 times in head length	1.8 to 2.3 times in head length	1.7 to 2.1 times in head length

Table 2. ***Meristic characters of commercially important groupers off the Kerala coast.***

Characters	*Epinephelus tauvina*	*E.malabaricus*	*E.diacanthus*	*E.bleekeri*	*E.chlorostigma*	*E.areolatus*
Dorsal fin spines	XI	XI	XI	XI	XI	XI
Dorsal fin rays	15-16	14-16	15 - 17	16- 18	16 -18	15- 17
Pectoral fin rays	18-19	18-20	17-20	17 -19	17 -19	17 -19
Pyloric caeca	16-18	Numerous	7-8	8-9	26-52	11- 17
Anal fin spines	III	III	III	III	III	III
Anal fin rays	8	8	8	8	8	8
Pre-opercle serrae/spines	Numerous fine serrae	I-VII	I-V	Numerous fine serrae	IV -VII	II -VII
Lateral line scales	63-74	54- 64	52 -60	49- 53	48 -53	49-53
Gill raker counts	8-10/17-20	23-27	8-10/ 15-17	25 -28	23 -29	Total 23-25
No.of colour bands/spots	Dark brown bnars with large brown and white spots on head, body and tail.	Body brownish, covered with small well-separated black apots; five irregular dark brown bars on the body	Pale grayish brown with five dark vertical bars broader than interspaces ; ventral part of head and body pink or reddish	Head and body brownish, covered with numerous gold or yellow spots lower two-third of caudal fin dusky	Head, body and fins with small irregular close-set dark brown spots. Caudal fin with white line along rear margin; spots on pectoral confined to rays.	Head,body and fins withnumerous yellowish brown or greenish yellow spots; posterior edge of caudal fin withdistinct white margin.

Table 3. ***Frequency distribution of Dorsal fin spines, Dorsal fin rays, Anal fin rays, Pectoral fin rays in commercially important groupers off the Kerala coast.***

Species	Dorsal fin spines					Dorsal fin rays						Anal fin rays				Pectoral fin rays					
	IX	X	XI	N	$\bar{X}$	14	15	16	17	N	$\bar{X}$	7	8	N	$\bar{X}$	17	18	19	20	N	$\bar{X}$
E.tauvina		2	18	20	10.9		8	12		20	15.6	1	19	20	7.95		5	15		20	18.7
E.malabaricus		2	18	20	10.9	2	6	12		20	15.5		20	20	8		2	5	13	20	19.5
E.diacanthus			20	20	20			11	9	20	16.45	2	18	20	7.9			7	13	20	19.6
E.chlorostigma			20	20	11			8	12	20	16.6	1	19	20	7.95	6	3	11		20	18.2
E.areolatus		2	18	20	10.9		2	16	2	20	16		20	20	20	2	5	13		20	18.5
E.bleekeri		1	19	20	10.95			15	5	20	16.25		20	20	20	8	7	5		20	17.8

Table 4. Frequency distribution of lateral line scales in commercially important groupers off the Kerala coast.

Species	Lateral line scales																				
	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	N	X
E.tauvina																	8	5	7	20	66.5
E.malabar										4		7		5	4					20	60.7
E.diacanth				8		8		3				1								20	53.8
E.chlorost	8	5			7															20	51.4
E.areolat	16	2		2																20	50.5
E.bleeker	2			8	8	2														20	51.4

Colour: Head, body and fins pale, covered with numerous dark brown, brownish yellow spots, those in front of head smaller than those on operculum; pectoral fins pale with small dark spots on rays. Posterior edge of caudal fin with a distinct narrow pale margin.

Distribution: Widely distributed along the continental shelves of Western Indian ocean, eastern Indian ocean, Red sea, and the Persian Gulf, east coast of Africa to the western, central Pacific; Oman and Cambodia, Indonesia, Philippines, Taiwan and Australia. Found in sea grass beds or on fine sediment bottoms near coral reefs and also on muddy bottoms at depths from 6 to 200m.

2.4. Discussion.

Classification and evolutionary relationships are important issues in the study of the groupers. The genus Epinephelus Bloch, 1793 is represented in tropical and subtropical latitudes of all oceans. They are among the most important commercial fishes of tropical fisheries of the world. This is the genus containing most number of species in the sub family Epinephelinae, comprising of around 98 species are the most top level predators on coral reefs . They are also among the highest priced fishes. A major predator of the coral reef ecosystem, most groupers feed on a variety of fishes, crustaceans and cephalopods. Adults of many are primarily piscivorous and are epibenthic predators. The large head and mouth of the typical grouper enables it to suck

in large volume of water and the prey together. The numerous inwardly depressible sharp teeth are well adapted for seizing prey and preventing its escape from the mouth.

Most of the species coming under this genus are found in the Indo-West Pacific region. Eight species occur in the eastern Pacific, eleven species are known from the Western Atlantic and nine species are found in the Eastern Atlantic and the Mediterranean. However, detailed information about the fishery is often not available; mainly because of the difficulty in correctly identifying the species. This is because of past taxonomic confusion, similarity in colour pattern of some species, ontogenic changes and other variations in colour pattern. Phenotypic identification of groupers of the genus *Epinephelus* is based on color patterns and a suite of morphologic characters. However, these characters often show intraspecific variations and differences between juveniles and adults of the same species. Recently, Govindaraju and Jayashankar (2004) attempted to study and ratify the status of *Epinephelus* spp. using random amplified polymorphic DNA (RAPD) analysis based on samples drawn from Southeast and Southwest coasts of India. The RAPD fingerprints were generated in *Epinephelus diacanthus*, *E. areolatus*, *E. chlorostigma*, *E. bleekeri*, *E. coioides*, *E. tauvina*, and *E. malabaricus* and found that *Epinephelus malabaricus* was most distantly related to *E. diacanthus* and *E. bleekeri*. The genetic relationship was very close among *E. coioides*, *E. tauvina*, and *E. malabaricus* and also between *E. chlorostigma* and *E. bleekeri*.

Attempts to elucidate evolutionary relationships among members of the genus Epinephelus have been hindered by the overwhelming number of species, pan-global

distribution and the lack of morphological specializations traditionally used in ichthyological classification. To date, no comprehensive phylogenetic study, morphological or molecular has been made to evaluate the monophyly of this genus. Most of the earlier works on systematics of Epinephelus were on the species from western Atlantic. Heemstra (1991) completed a taxonomic revision of 14 species of groupers in the Eastern Atlantic Ocean and the Mediterranean Sea; Randall and Heemstra (1993) revised the taxonomy of Indo-Pacific groupers. Smith (1971) assigned the eastern Pacific species *Bodianus acanthistius*, though this has only IX dorsal-fin spines, to the genus Epinephelus rather than to Cephalopholis. Smith (1971) revised the American groupers- Epinephelus and allied genera, demoted the genus Promicrops to a subgenus of Epinephelus. Randall and Ben Tuvia (1983) gave the systematics on the groupers (Epinephelinae) of the Red Sea. According to Smith-Vaniz *et al.*, (1988), the scales of Alphestes and Dermatolepis are different from all other groupers. Phylogenetic relationships among epinepheline genera were investigated based on cladistic analysis of larval and adult morphology (Baldwin and Johnson 1993). Smith (1971) also hypothesized that Alphestes is a descendant from Epinephelus, but the genus Myctoperca was not ancestral to it and also that Grammistes and Plectropomus were sister taxa.

Several conclusions drawn from the DNA sequences analysis (Ding *et al.*, 2006) were (1) genus *Plectropomus*, which was early diverged, is the most primitive group in the subfamily Epinephelinae; (2) genus *Variola* is more closely related to genus *Cephalopolis* than the other four genera; (3) genus *Cephalopolis* is a monophyletic group and more primitive than genus *Epinephelus*; (4) *Promicrops lanceolatus* and *Cromileptes*

altivelis should be included in genus *Epinephelus*; (5) there exist two sister groups in genus *Epinephelus*. Ding *et al.*, (2006) studied the cytochrome *b* gene fragment of twenty-eight grouper species within six genera of subfamily Epinephelinae, amplified using PCR techniques and the sequences were analyzed to derive the phylogenetic relationships of the groupers from the China Seas

Phylogenetic systematics and patterns of ecomorphology defined as variation in morphology that contributed to variation in feeding ability among the groupers are poorly known. An early paper by Randall (1967) briefly mentioned differences in dentition and feeding behavior of certain piscivorous groupers, but further investigations of feeding morphology in epinepheline serranids have not been undertaken. According to Baldwin and Johnson (1993) larval features provide evidence of a monophyletic Epinephelini.

Chapter III.

Female reproductive system and the process of oogenesis in *Epinephelus tauvina.*

3.1. Introduction

The gonads of all vertebrates have double walled origin, ovary developing from the more laterally located cortical portion. The medullary portion that is destined to form the testis arises from more medial cellular proliferation. Usually, one of these portions grows rapidly while the other fails to develop and the sex of the individual is thus determined at a very early stage. Though this pattern of gonad differentiation is characteristic of elasmobranchs, the gonads of teleosts and cyclostomes develop from single primordia, directly in the peritoneal epithelium underlying the genital ridge and corresponds only to the cortex. (Hoar and Randall 1969, Brusle 1987, Brusle-Sicard *et al.*,1992, 1994,

Guraya 2000). It has been suggested that this may account for the more widespread occurrence of intersexuality among cyclostomes and teleosts. Basically the morphology of germ cells and the different somatic cell elements constituting the teleosts gonadal tissue are similar although a complexity of reproduction with varying gonadal structures exists in different species (Nagahama 1983).

The problem of reproduction and the histophysiological studies of the gonads and their seasonal changes have been the subject of investigation by a large number of workers. Basic information on the morphology, anatomy, physiology and cytology of the reproductive system of a candidate species is most essential to develop biotechnological methods for controlled induction of reproduction, to obtain predictable supply of quality seeds of known heritage, better stock and increased yields. With the wide-spread practices of intensive farming of marine fishes, knowledge of the reproductive processes along with the many factors controlling their reproduction has become very much imperative. Cytological studies correlated with morphology during the process of ovarian maturation form a basic tool for the assessment of maturity in females. Understanding of reproduction of the candidate species requires combined knowledge of all aspects viz. the changes in the morphological characters of gonads and corresponding cytological features, which are essential for the better management of brooders for seed production. Such studies are also essential in proper staging of reproductive phase of animals in hatcheries, as precise staging of the gonadal maturation is essential for the selection of females for breeding and production of seeds for farming.

According to Sadovy (1995), a better understanding of grouper reproductive biology would greatly facilitate stock management. Information on annual fecundity, sexual maturity, number of spawns and age and size at maturity are useful in management (Collins *et al.*, 1996); the causes of sex change in groupers must be analysed to estimate how fishing activity could influence the population's reproductive output and to determine the effects of protogynic hermaphroditic reproductive strategy on management measures (Shapiro 1987).

The pattern of changes in the gonads during reproductive cycles varies from one species to another. The actual developmental stages of growing oocytes cannot be determined macroscopically; it is also difficult to distinguish the presence of atretic follicles in the ovary and also distinguish between the maturing virgins from the recovering spents, macroscopically. The transitional stages and immature stages of males of groupers are also not distinguishable macroscopically. Therefore, to understand their reproductive physiology, a detailed histological study, which provides very precise information on the gametogenesis, is necessary. Descriptions of the different stages of oocyte growth and development have been given by Yamamoto (1969), Moe, (1969), Guraya (1994) and West (1990).

Smith (1965) states that gonads of all hermaphroditic serranids are similar in gross appearance. *E.tauvina*, a protogynous hermaphrodite, does not exhibit any externally distinguishable sexual characters. Females had a higher GSI than androgen-induced males. The functional testis is smaller than ovary in this species. The abdomen of the

female fish becomes flabby during spawning period due to the enormous increase in size of the ovaries. Ovarian and testicular tissues were not separated by connective tissue, but these two tissues or female / male germ cells were intermixed during the course of controlled sex change, characteristic of the undelimited gonad type 2 (Shapiro,1987). In ***E.tauvina*** ovaries are of cystovarian type the ovaries are paired hollow sacs lying in the body cavity. The two lobes join together posteriorly to form a common oviduct which open to the exterior immediately behind the anus.

The production of eggs, well equipped with the necessary reserve food for the developing embryo, takes place through a process within the developing germ mother cells of the ovary. The present study has been carried out to understand the events occurring in the ovary of the female during the process of oogenesis. Investigations on the changes in gonad development in fishes are many; to cite a few are those of West (1990), Bromage (1995), Yamamoto and Yoshioka (1964); Wallace and Selman (1979); Treasurer and Holliday (1981); Qasim (1973); Nagahama (1983); Mayer *et al.*, (1988); Le Cren (1951); Hickling and Rutenberg (1936); Devaraj (1983), Forberg (1982); Issac-Nahum *et al.*, (1988) etc. In serranids and related groups, studies using light microscopy, those of Bouain and Siau (1983) Lee *et al.*, 2002, Chen *et al.*, 1971, Yeh, 1986; Yeh, 2003, Chao and Lim (1991), Johnson *et al.*, 1998, Abu-Hakima 1982, Yamomoto (1969); Glamuzina *et al.*, 1998; Kuo, 1988; Lee *et al.*, 1995; Quinitio (1997), Shapiro (1987), Tan-Fermin, 1994, Tan and Tan (1974) Shapiro *et al.*, 1993, are of special relevance to the present study. The present investigation includes the classification of the ovarian maturity stages based on colour, gonadosomatic index, oocyte diameter and

morphological changes taking place within the oocyte. The process of oogenesis has been investigated using light microscopic techniques.

3.2. Materials and methods

In the present study, the method of classification of maturity stages for ovary followed is that used by Thompson and Munro (1978) in the study of maturity stages of serranids from the Caribbean sea. Various stages of maturity of female gonad ie. the ovary, were taken from fish samples collected from the wild.

3.2.1 Collection of fishes

The samples for the study were collected mostly from among the groupers caught by the Chinese dip nets operated at Cochin bar mouth and the nearshore areas. During sampling the surface water temperature, salinity and Dissolved Oxygen were also taken from the sampling site. Salinity was determined using salinometer/conductivity meter (make: Atago). Water samples for dissolved oxygen estimation was collected without agitation and fixed with Winkler's reagents as per the standard procedure, which was later determined by the titration method (Strickland and Parsons 1968), in the laboratory **(Table.5).**

3.2.2 Dissection and fixation

The collected fishes were cleaned well and sorted out according to size. After blotting out the water adherent on them, each fish was weighed to the nearest milligram, total length and standard length of fishes were measured to the nearest millimeter . The

Table 5. *Monthly variation in water temperature, salinity and dissolved oxygen at Cochin bar mouth and near shore area during 1998-1999.*

Month	Mean Temp (^{o} C)	Mean salinity (ppt)	MeanD.O (ml/L)
1998			
Jan	29.5	31.20	3.11
Feb	31.0	31.0	3.78
Mar	32.0	33.50	4.00
Apr	31.0	32.44	4.12
May	31.8	34.82	3.76
June	30.1	30.21	4.84
Jul	28.5	16.63	7.02
Aug	29.0	10.10	6.43
Sept	31.0	21.28	5.51
Oct	30.0	20.35	6.39
Nov	29.8	22.12	5.24
Dec	30.2	29.99	3.83
1999			
Jan	28.0	33.29	3.25
Feb	31.5	31.20	3.60
Mar	32.0	33.0	3.80
Apr	31.6	31.45	3.66
May	32.0	29.45	2.20
Jun	28.5	10.00	3.05
Jul	26.3	16.05	5.42
Aug	26.5	28.50	8.05
Sept	26.5	29.66	6.11
Oct	28.5	19.36	5.38
Nov	29.5	20.30	4.95
Dec	29.0	29.48	3.85

abdomen was cut open to expose the ovary; gross morphological observations of the ovary and oviducts were made. Colour, shape, length, breadth and weight of the gonad and its volume in relation to the body cavity were recorded; it was then carefully excised and removed. Based on the volume occupied by the gonads and the general macroscopic appearance, they were assigned to different maturity stages using six stage maturity scale. For carrying out the histological studies, pieces from anterior, middle and posterior portions of each lobe of the ovary were cut and fixed in Bouin's fixative for 24-48hrs.

3.2.3. Processing and Sectioning.

All the tissues fixed in Bouin's fluid were washed in running tap water to remove excess picric acid. Both Bouin's and formalin fixed tissues were dehydrated using alcohol series (30% t0 100% ethanol) and cleared in methyl benzoate. The tissues were further cold impregnated overnight with wax using benzene and wax shavings in a 1:1 ratio. Subsequently the solvent was evaporated by placing the tissues in an oven at 58° C.The tissues were then transferred through two changes of fresh molten wax (Paraffin wax with ceresin, BDH (58-60° C). Tissue blocks were prepared by using paper boats or small glass troughs after proper orientation. Serial sections of the blocks were cut approximately 6-8µ thickness on a rotary microtome using disposable blades. Sections were affixed on clean glass slides using fresh Mayer's egg albmin and flattened by placing on a slide warmer with a drop of distilled water. Subsequently the water was drained off and the slides were allowed to dry. These slides were then stained for histological studies.

3.2.4. Staining

Staining was carried out using (Heidenhain's) haematoxylin stain with 1% aqueous eosin as counterstain. Sections to be stained were first deparaffinised in two changes of xylene and then hydrated through a down series of ethanol grades. Sections were blued using tap water and 1% lithium carbonate. Eosin stained sections were repeatedly washed in 95% alcohol to remove the excess eosin. Slides were further dehydrated in absolute alcohol and cleared in xylene and mounted with DPX or Canada balsam of neutral pH. Mounted slides were examined under a light microscope.

3.2.5. Micrometric measurements.

The micrometric measurements of the oocytes in different stages of maturation were taken using an ocular micrometer calibrated with a stage micrometer. As a result of oogenesis, the oocytes increase in size, and also deviate fron the typical spherical shape, the largest and the smallest axes of the oocyte diameter was taken and the average was used as the actual oocyte diameter.

3.2.6.Photomicrography.

Photomicrographs of the histological preparations of the ovary were taken using a Leitz binocular microscope equipped with an automatic exposure system using 35mm colour (KODAK COLOUR 100 ASA) film.

3.3. Results

3.3.1. Gross Anatomy of the Ovaries

The ovaries of *E.tauvina* are paired cylindrical compact hollow structures, lying in the posterior abdominal region of the body cavity ventral to the kidney, below and behind the posterior part of the air bladder and connected with it by mesenteries. The gonads of *E.tauvina* are relatively small, the weight of the ripe gonad relative to the total weight of the fish is small. Both the lobes of the ovary are separable and closely opposed to each other throughout their length. Each lobe of the ovary is covered externally with a thick peritoneal layer comprising of connective tissue, smooth muscles with blood vessels entering dorsally via the mesorchium. There is an innermost layer of germinal epithelium which projects into the ovocoel forming lamellae. The oogonia are present in clusters in these ovigerous lamellae. As maturity advances, the ovocoel gets obliterated due to increasing number of ova.

Determination of reproductive state of the animal is of utmost importance for fishery management as well as in carrying out culture programmes. In *E. tauvina* as in other fishes determination of ovarian maturation is possible through evaluation of colour changes in the ovary during maturation and increase in the ovarian volume ie. GSI, during maturation. In young groupers, early stage ovary was observed as pinkish white with thick connective tissue strands. Ovary in advanced stages was creamy or whitish creamy in colour, larger, the ovarian wall was thinner, ovary taff with egg mass inside. When oocytes were fully mature or ovulated it could be seen clearly through the ovarian wall and ovary was softer. During the breeding period, the gonado-somatic index rises much. There is increase in the ova diameter.

Ovaries in groupers always developed towards the right side of the fish body. The two lobes of the ovary are attached to the body wall by means of mesovarium. The right and left lobes of ovary of *E.tauvina* are unequal; the right lobe is bigger than the left one by 10-12%. The two lobes are joined posteriorly, each lobe is covered by a thick muscular tunica; caudal end of each ovary forms a short thick walled oviduct and the two oviducts fuse and open to the exterior by a common urinogenital opening immediately behind the anus opening. The anterior free ends of the ovaries are rounder than the posterior end. The maximum length of the right lobe in ripe condition was about 22-25cm. Supporting mesentries continue forward from the anterior end of each gonad and join as a complex of ligaments and mesenteries at the anterior end of the swimbladder. In the early growth phase, the gonads are flaccid, delicate and transluscent structures, pale beige in colour occupying a small volume in the visceral cavity. The ovaries turn yellowish pink in colour, becoming enlarged and distended during maturation and contain oocytes, which are clearly visible through the gonad wall. During fully mature reproductive period, the genital papillae of females are distended and vascularized.

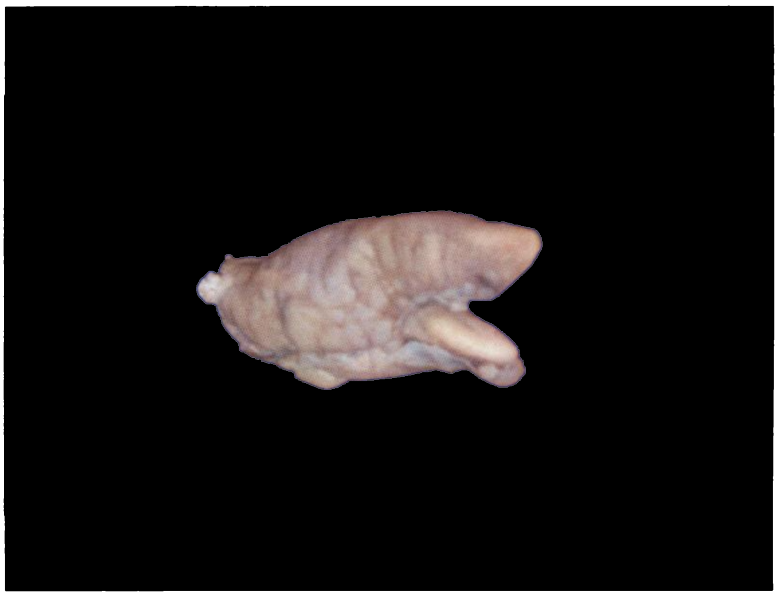
Histologically, the outer part of the ovary is the thin peritoneum, followed by a layer of tunica albuginea, which is made up of connective tissue, muscle fibres and blood capillaries. There is the thin innermost layer of germinal epithelium which projects into the ovocoel and forms several lamellae. These lamellae are the seat of primordial germ cells, which probably originate from the inner part of germinal epithelium.

3.3.2. Maturity stages:

Table. 6. ***Classification of maturity stages in female phase, based on colour of ovary, GSI and Oocyte diameter.***

Ovary stage	Colour and appearance	GSI	Mean Oocyte diameter	Histological features
Stage I Immature ovary with Primary oocyte	Small, thread like, transluscent and flesh coloured	-	15 to 20μm	Ovary small with compact lamellae, each containing pre-vitellogenic oocytes, active zone of proliferation with clusters of developing oogonial cells; atretic bodies absent, gonad wall is thin
Stage II maturing virgins or spent recovering; immature oocyte	Pale yellow, granular and smooth	0.073	60 to 100μm	Nucleus with several nucleoli, ; nucleoli present around the nuclear periphery
Stage III Maturing phase	Yellowish and cylindrical, granular	0.154	180-280 μm	Oocytes in chromatin nucleolus phase,oil globules accumulate in the cytoplasm, giving it granular nature.
Stage IV Ripe, pre-spawning phase	Massive large golden yellowish,granular ovaries	0.364	375-450 μm	Rough granular cytoplasm, follicle cells considerably stretched.Yolk abundant.
Stage V Ripe,oozing, spawning phase.	Massive large golden yellowish ovaries with ova loosely packed.	4.571	400-900 μm	Oocytes increase in volume due to hydration, yolk globules form homogenous mass, more transluscent.
Stage VI Spent ovary	Ovary shrunken and flaccid, bloodshot and transluscent.	0.454		A few opaque, disintegrated oocytes are seen, also immature oocytes measuring 20-80 μm also seen.

PLATE IV



a. Photograph of early maturing stage of ovary of *Epinephelus tauvina*

b. Photograph of maturing phase of ovary in *E.tauvina*

One of the convenient methods of determining the reproductive cycle in fishes is to study the developmental changes in the gonads through macroscopic observations. In *E.tauvina* juveniles do not exhibit internal differentiation of sex; among fishes measuring 38-40cm in total length, the females can be distinguished by the presence of pinkish, translusccnt, ribbon-like strands of gonadal tissue. Maturity stages have been identified mainly based on the size, shape, colour and texture of the ovaries and also on microscopic structure of the ova **(Table 6).**

a) **Stage I:** Immature phase: The ovaries are very small, flesh coloured, thread like and transluscent measuring about 1-3 cms in length with no evidence of past spawning. The gonads are compact with tightly aligned lamellae and nests of oogonia. Vascularisation at this stage is inconspicuous. The transparent oocytes embedded in connective tissue, under magnification appear transparent, irregular, with a central nucleus, and without yolk. Oocytes measure only 15μm to 20μm.

b) **Stage II**: Maturing virgins or recovering spent: (**Plate IVa**) Both virgin as well as spent recovering females are included in this category. The two lobes of the ovary are unequal in length, measuring 2-5cm in length and 0.5 to 1.0cm in width. A few small ova are visible under microscope; they appear white to pale yellow in colour, granular, not fully rounded; the process of yolk deposition is seen; nucleus is seen centrally located. There is increase in size and weight of ovary. Oocyte diameter ranges from 60μm to 100μm, with mode at 80μm. A few atretic follicles are present in recovering spents. (Recovering spents cannot be distinguished macroscopically, but histologically a

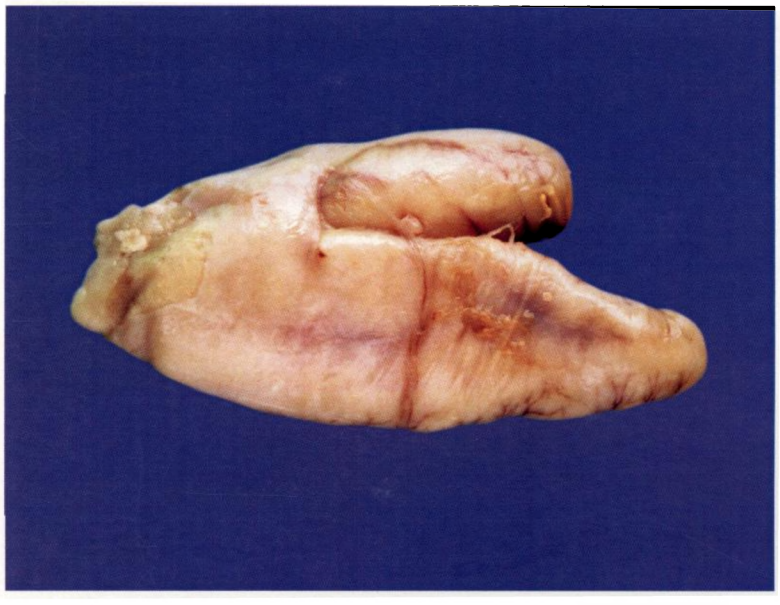
recovering spent ovary is characterized by having thicker ovary wall, and often contain residual atretic oocytes) Fishes measuring 400mm or more in total length possessed ovaries in this stage.

c) **Stage III:** Ripening or Maturing phase: **(Plate IVb)** The ovaries attain large size with marked increase in weight and volume; each ovarian lobe becomes almost cylindrical, characteristically yellowish; the two lobes are unequal in length with the right lobe longer than the left and measure 6 to 8cm in length and width 1.5 to 2.5cm; vascular supply prominent on the surface of the gonad, transluscent and packed with small eggs . Ova are yellowish, granular, round, semi opaque and incompletely yolked. The ovigerous lamellae are very much swollen and laden with oocytes. Yolk deposition is also seen. Oocyte diameter reaches 180- 200μm.

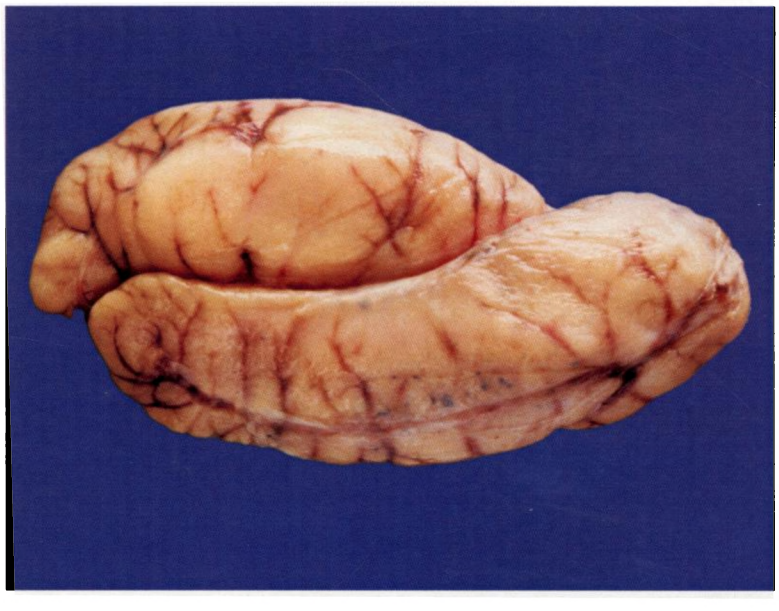
d) **Stage IV:** Active, ripe or pre-spawning phase: **(Plate Va)** The massive golden yellowish ovaries are large and the tunica albuginea thin; fully packed with numerous ripe ova occupy about 2/3 of the body cavity, the ovaries fully vascularized, ovarian wall becomes very thin and the spherical ova seen by naked eye; the ripe ova are transluscent, measure 375 - 450μm in diameter, double walled and possess yolk granules and a single oil globule; another group of smaller yolked ova are also present. This is seen to be a rapid phase of growth and development.

e) **Stage V.** Ripe or spawning phase: **(Plate V b)** The gonads are massive and greatly enlarged, occupying the entire visceral cavity, measuring 20-25cm in length and 8-10cm

PLATE V



a. Photograph of a ripe, pre-spawning ovary of *E.tauvina*



b. Photograph of a ripe and oozing ovary of *E.tauvina*

in width; almost all eggs are loosely packed, under microscope they appear transparent, double walled with a single large oil globule inside. At this stage ova can be extruded if a gentle pressure can be applied on the abdomen. The ovaries are golden yellow in colour and wall very thin and almost very transparent. The hydrated and released ova measure 400-900 μm in diameter, with mode at 820μm and possess a single oil globule. Eggs were present in the oviducts also.

f) Stage VI: Spent phase : The shrunken, flaccid ovaries occupy almost half of the body cavity; they appear blood shot, transluscent, loosely packed with microscopic and transparent primary oocytes; blood vessels are prominently seen on the surface; a few large, opaque, disintegrated yolky oocytes are also observed, which undergo the process of resorption; the ovary measures 12cm in length and 2.5cm in width. Immature oocytes measuring 20μm to 80μm in diameter with prominent mode of 50μm was observed in stage VI ovary.

3.4. The process of Oogenesis

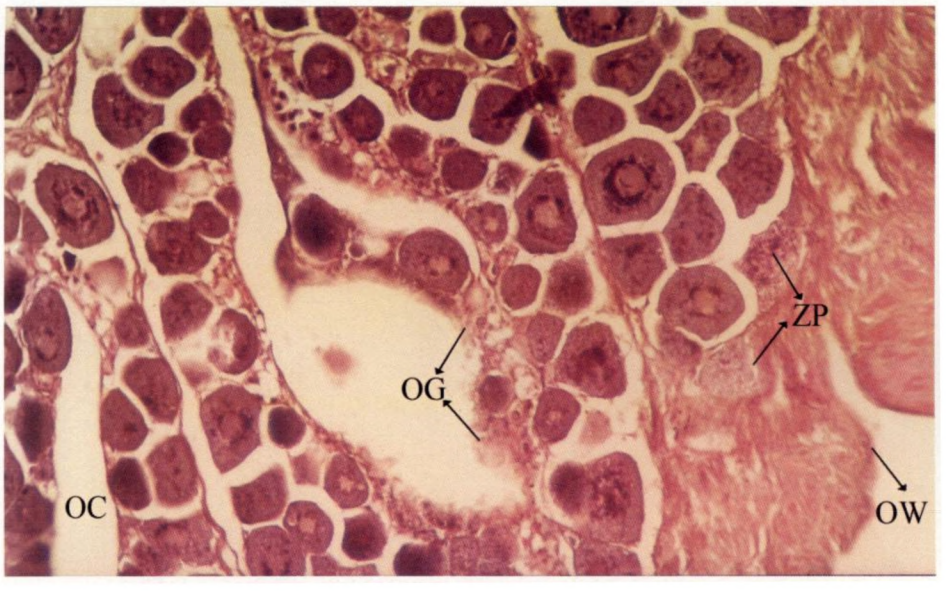
The production of egg, well equipped with the necessary reserve food for the developing embryo, occurs through the processes taking place in the germ mother cells of the ovary. These cyclical processes include a series of events starting from activation of primordial germ cells to the differentiation of highly yolk-equipped ova. The oogonial cells transformed themselves into mature ova with sufficient yolk for the development of the embryo. Clear knowledge of the precise stage of gonadal maturation is highly essential for the selection of female spawners for breeding and seed production.

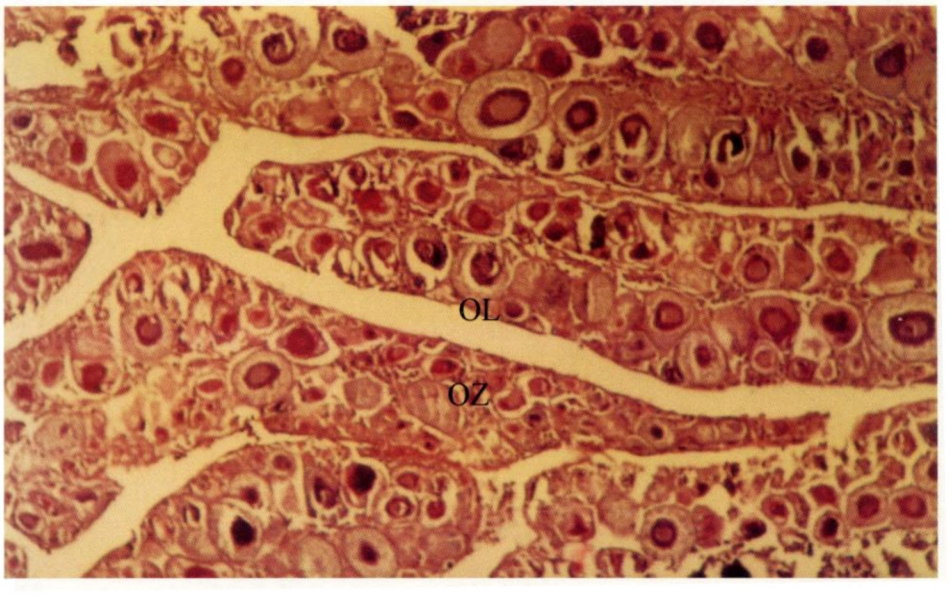
Cytological studies correlated with morphology during the process of ovarian maturation are essential in correct staging of reproductive phase of candidate species in hatcheries.

Light microscopical examination of the histological sections of the ovary in different stages of maturity revealed the process of oogenesis and the manner in which oocytes developed and accumulated yolk. Based on the changes taking place in the cytoplasm as well as the nucleus of the oocytes, the developmental stages were classified into different phases viz. immature, pre-vitellogenic, mid–vitellogenic, vitellogenic, oocyte and spent stages . These phases of the oocyte correspond to the stages I to VI described earlier based on morphological characters.

Sections of the ovary show that it was encompassed by a thin ovarian wall, consisting of two distinctive layers; a thin outer layer of epithelium, which was moderately basophilic with haematoxylin and eosin and the inner layer of relatively loose connective tissue which was eosinophilic (**Plate VI a**). Blood capillaries were also observed on the ovarian wall. A germinal zone was observed as a thin band along the innermost layer of the ventolateral periphery of the ovarian wall. This **"zone of proliferation"** from which the displacement of oogonial cells takes place, was seen to persist in all maturity stages. It can be observed that young oocytes moved from the germinative zone upon maturation, so that the developing oocytes and ova were found towards the center of each ovarian lobe in a graded manner (**Plate VI b**). The characteristic feature in the immature stage is the presence of clusters of developing

PLATE VI





Photomicrograph of section of ovary showing ovarian wall (OW), ovarian cavity (OC), ova at the center of the ovarian lamellae (OL) and young oocytes (OZ) and oogonia (OG) at the zone of proliferation (ZP). X100

oogonial cells in the active proliferation zone. The primary and secondary oogonial cells were also seen with the secondary oogonial cells shifted to the interior.

3.4.1 Immature stage :

The characteristic features seen here in a light microscopic examination are the active zone of proliferation with clusters of developing oogonial cells(**Plate VI a**). The primary and secondary oogonial cells are arranged in graded manner in the ovary so that the growing secondary oogonial cells are shifted to the interior . By mitotic division of the primary oogonial cells the secondary ooogonial cells which are larger than the primary oogonial cells are formed. The oogonial cells are crowded together in the narrow germinal zone, the cytoplasmic boundaries often ill-defined and the follicle cells apparently absent in this stage.

Primary oocytes are formed by reduction division of the secondary oogonial cells. These cells appeared round and possessed a large and conspicuous nucleus occupying almost 80% of the cell volume; the cytoplasm was palely eosinophilic. The nucleus stained with haematoxylin; and the chromatin matter distributed uniformly. The entire cell is stained deeply with haematoxylin. The central ovarian cavity and lamellae are seen(**Plate VI b**). During the immature phase, the nucleus of the oocyte had a large central nucleolus and several smaller nucleoli around the nuclear periphery.

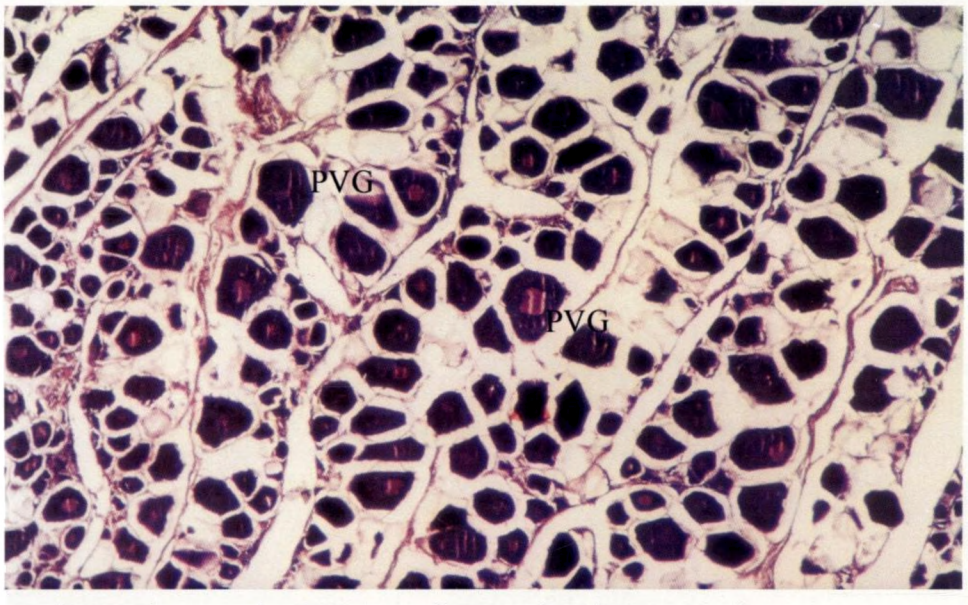
3.4.2 Primary or pre-vitellogenic oocytes: (Plate VIIa)

The primary oocytes formed by meiotic division of the secondary oogonial cells, were found in groups closely associated with the membrane of the lamellae and occupied

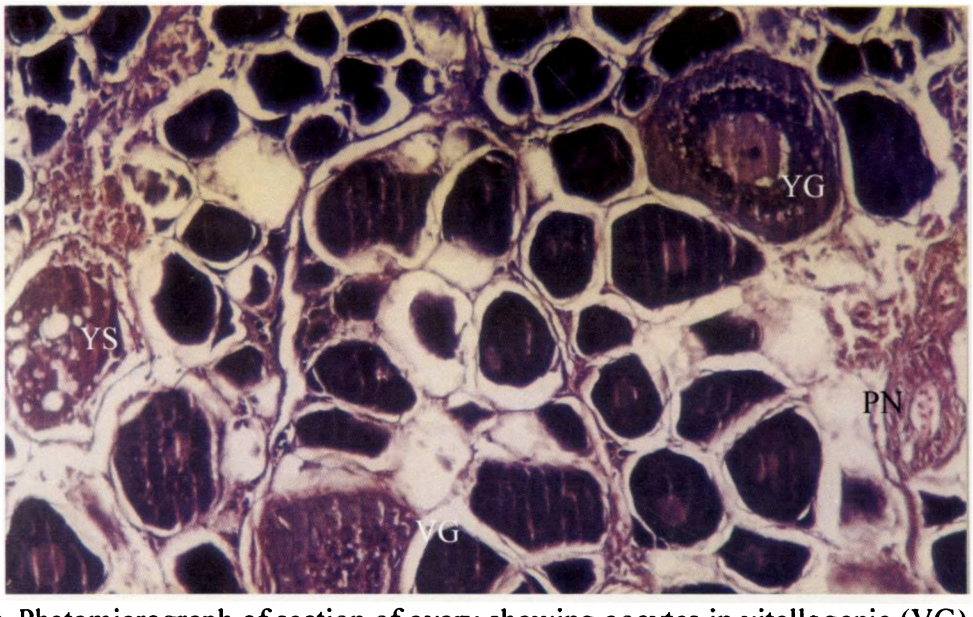
the entire lumen in clusters surrounded by follicle cells. The early vitellogenic oocytes were round to oval with a sudden increase in size noticed . The nucleus increased in size reaching a diameter of 30 to 50 μm and contained multiple nucleoli arranged as a circular ring around the periphery of the nucleus. A sudden increase in the cytoplasmic volume was noticed and from this stage onwards the oocytes started accumulation of yolk. Pre-vitellogenic oocytes were highly basophilic; there is increase in the cytoplasmic volume and measured upto 15 ±2.8 μm.

Oocytes at the primary growth phase correspond to stages I and II in serranids (Abu-Hakima 1987). Two phases could be observed in this stage ie. oocytes in chromatin nucleolus phase and oocyte in perinucleolus stage; the former are smaller while the latter appeared round to oval cells with prominent nucleus. The large nucleus was stained lightly with haematoxylin and contained a single deeply stained nucleolus and strands of chromatin along the peripheral margin. In the early stage of this phase the cell dimensions ranged from 40- 180 μm; the large nucleus measured 30μm in diameter with a single nucleolus at the early stages of primary growth phase. The cytoplasm was basophilic. Later, as the oocyte increased in size, the nucleus increased in size reaching a diameter of upto 50μm and contained numerous darkly stained nucleoli and lampbrush chromosomes. The nucleoli are arranged as a circular ring around the peripheral margin of the nucleus. The perinucleolar oocytes were identified by the displacement of the nucleoli towards the periphery of the nucleoplasm. The cytoplasm now appear granular due to the presence of vesicular primary yolk. A follicular epithelium is also present around each oocyte.

PLATE VII



a. Photomicrograph of section of ovary showing previtellogenic oocytes (pvg) of different size along the ovigerous lamellae X100



b. Photomicrograph of section of ovary showing oocytes in vitellogenic (VG), perinucleolar (PN) and yolk vesicle (YS), yolk granule (YG) stages. X200

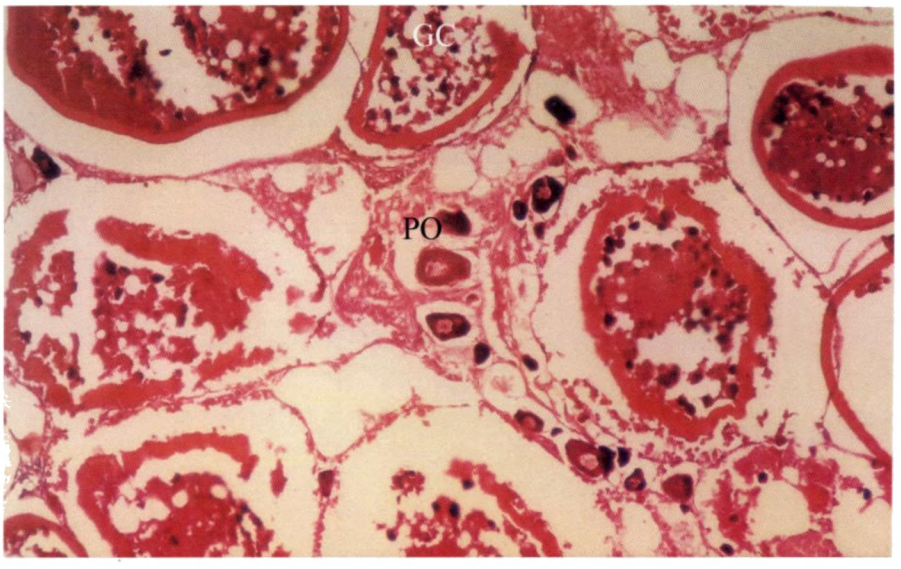
3.4.3 Early-vitellogenic oocytes: (Plate VIIb)

Oocytes in the early stages of vitellogenesis, corresponding to stage III oocytes (Smith,1965; Moe,1969; Abu-Hakima 1987) was characterized by a marked increase in size. The early vitellogenic oocytes were round to oval with a sudden increase in size noticed reaching upto 200 – 280 μm in diameter. The cytoplasm also changed suddenly from homogenous to vesicular. The nucleus increased in size reaching a diameter of 30 to 50 μm and contains multiple nucleoli arranged as a circular ring around the periphery of the nucleus. During secondary growth phase, lipid droplets, protein yolk globules and cortical alveoli are formed in the oocytes. These protein yolk globules appeared first in the cortical cytoplasm and later filled the entire cytoplasm. A sudden increase in the cytoplasmic volume was noticed and from this stage onwards the oocytes started accumulation of yolk. Oil globules accumulated in the cytoplasm giving it granular nature which is a characteristic feature of early vitellogenic oocytes. The nucleolar materials made a halo around the nucleus due to their circular arrangements in the peripheral karyoplasms. Formation of follicle cells around individual oocyte was also complete dring this stage; the follicle cells stretched considerably and consequently decreased in thickness. Some oocyte-like cells, without nucleus (pseudo-oocytes) was also observed among growing oocytes. These pseudo-oocytes became reduced in size as the neighbouring oocytes increased in size.

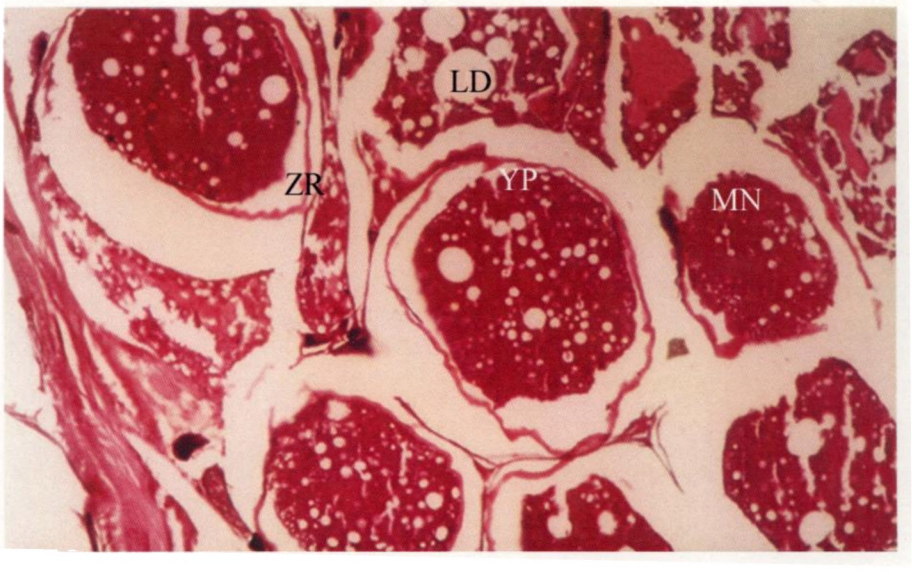
3.4.4 Vitellogenic oocytes: (Plate VIII a&b).

In stage IVand V ovaries, the ovaries are filled with vitellogenic oocyte measuring upto 425 ± 10.6μm and their nucleus upto 60 ± 3.4μm in diameter. A characteristic

PLATE VIII



a. Photomicrograph of vitellogenic oocyte stage with granular cytoplasm (GC), formation of follicle cells and presence of pseudo oocytes (PO)X100



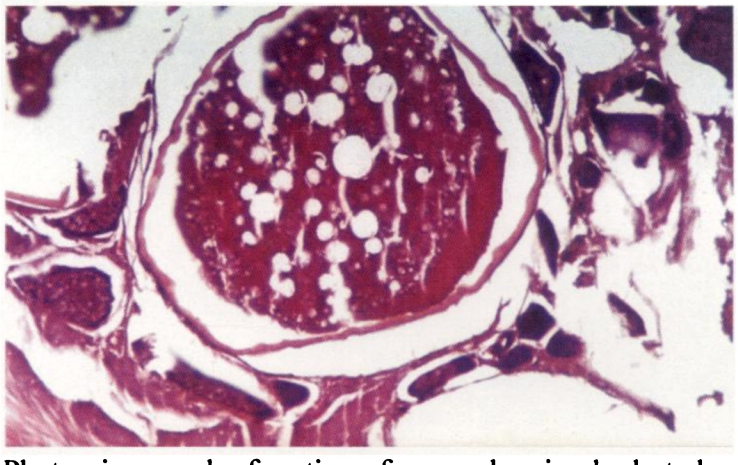
b. Photomicrograph of late vitellogenic oocytes showing yolk platelets(YP), migrating nucleus (MN) lipid droplets (LD) and zona radiata (ZR) X100

feature of this phase was the rough granular cytoplasm, which has lost its basophilia. The granular nature of the cytoplasm was mainly due to the formation of dense yolk platelets and accumulation of lipid droplets. Platelet phase oocytes possessed a highly vacuolated, rough, granular type cytoplasm that was wholly eosinophilic on staining. The lipid droplets were not stained. The fully formed yolk platelets were dark blue in colour with toludene blue stain. The follicle cells were considerably stretched. The nuclear membrane showed a convoluted form, stained faintly, with numerous small nucleoli lying at the periphery. The ooplasm is now largely replaced by the acidophilic yolk globules, which coalesce and become large and well defined. The lipid droplets continue to increase in size with the larger ones lying near the nucleus. The zona radiata (ZR) appears as a thin acidophilic band between the oocyte and the overlying follicular cells.

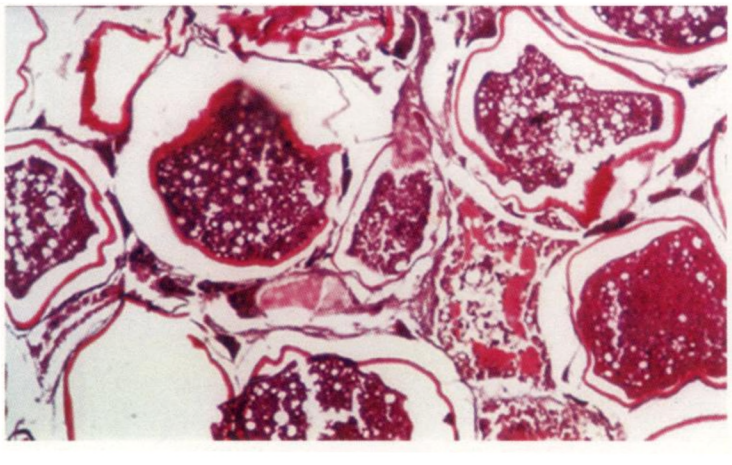
The follicle cells were further flattened and were present only as a thin covering. The matured oocytes appeared more elongated than circular with a very thin rim of follicle cells around it. Vitellogenic oocytes are characterised by the abundance of mature yolk. The nucleus became greatly reduced in size, lost its round shape due to the disintegration of the nuclear wall, migrated to the periphery. During late vitellogenic stages, the germinal vesicle shifts to an eccentric position in the cytoplasm and yolk fusion commences. The cytoplasm and the follicle cells were wholly eosinophilic upon staining.

3.4.5 Oocyte stage (Plate IX)

PLATE IX



a. Photomicrograph of section of ovary showing hydrated ova, the homogenous yolk globules also seen X200



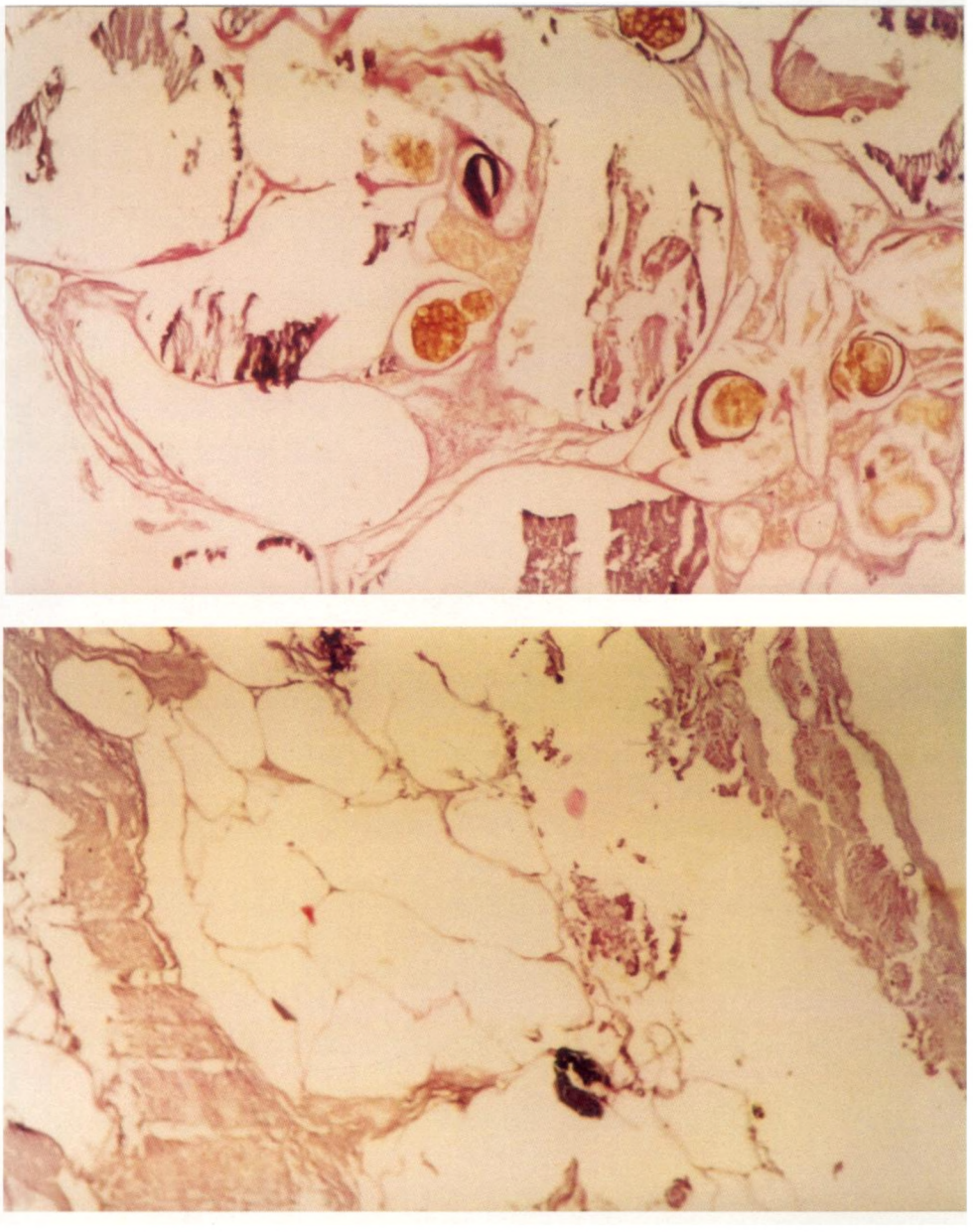
b. Ovary in spent stage with regressing oocytes X100

The oocytes rapidly increase in volume due to hydration; the hydrated and released ova measured 400-900 μm in diameter, with mode at 820μm; the yolk globules form a homogenous mass in the ooplasm, becoming more transluscent. Lipid droplets also increase in size and the single largest one at the center measure100μm. The follicular layer becomes stretched due to increase in cell volume. At this stage the oocyte resumes meiotic division and are released into the ovarian cavity (ovulation) by rupturing the follicular wall and finally to the outside (spawning).

3.4.6 Spent oocytes: (Plate IXb, X)

Microscopically the spent ovaries showed the disintegrating empty follicles from which ova have been released, surrounded by invasive cells which gradually resorb them; attretic bodies were observed near to the connective tissue of the ovary and also near to areas of proliferative growth of oocytes at this stage. Empty follicles from which ova have been released have hypertrophied follicle cells. Pycnotic oocytes having deeply basophilic cytoplasm and nucleus were also observed in conjunction with hypertrophied follicle cells. The residual oocytes in various stages of development and differentiation, remnants of yolk globules, yolk vesicles and the empty follicles are lost from the ovary by the process of oocyte atresia. These atretic bodies form a characteristic brownish mass, the corpus atreticum, composed of amorphous brownish granules, phagocytes and yellow pigment globules (Moe, 1969). Unshed stage IV oocytes, unlike early stage III oocytes cannot regress to resting eggs, but have to be resorbed. The zona radiata is quickly lysed, and the follicles contained only yolk vesicles, yolk globules and undifferentiated ooplasm. The yolk globules become granular and dispers in the lamella

PLATE X



Ovary in atresia stage and pycnotic oocytes
with hypertrophied follicle cells X100

and the undifferentiated ooplasm gets resorbed. Atretic bodies formed at the site of degenerated oocyte, eventually move into the center of the ovarian lamella.

These had also characters similar to the pre-vitellogenic or early vitellogenic stages and in the post spawning gonad, ovarian tissues undergo reorganization. Zone of proliferation with darkly stained irregular shaped fresh batches of primary oogonial cells and developing oocytes with characteristics of pre-vitellogenic oocytes was also observed at certain portions of the spent ovary. Pycnotic oocytes which have basophilic cytoplasm and nucleus were also observed in conjunction with hypertrophied follicle cells.

3.5. Gonado-somatic index

Reproductive cycles are characterized by pronounced variations in gonadal size. When assessing gonadal activity, animals of different sizes are frequently sampled and it is generally assumed that gonadal weight depended on the size of the animal and stage of gonadal development. In fishes, the GSI is widely used as an index of gonadal activity and as an index for spawning preparedness. The most common means of accounting for the effects of differential body size on gonad size has been to express gonadal weight as a percentage of body weight. This ratio of gonadal weight and body weight (gonadal weight/ body weight x100) is termed the gonadosomatic index (GSI). The gonadal index is useful for separating the spawning from the non-spawning fish. While assessing gonadal activity, animals of different sizes are frequently sampled and it is greatly assumed that gonadal weight depends on animal size and stage of gonadal development.

Table 7. ***Macroscopic and histological characteristics of female developmental stages in E.tauvina.***

Developmental stages	Symbol	Defining characteristics
lmmature female	F1	Ovary small with compact lamellae, each containing pre-vitellogenic oocytes(ie. chromatin nucleolus and perinucleolus stages), active zone of proliferation with clusters of developing oogonial cells; atretic bodies absent, gonad wall is thin.
Pre-vitellogenic female	F2	Lamellae less compact, contains oocytes of same stage surrounded by follicle cells. Atretic brown bodies present.; gonad wallis thick
Early vitellogenic stage	F3	Ovary with vitellogenic oocytes; sudden increase in the cytoplasmic volume, the oocytes started accumulation of yolk. Oil globules accumulate in the cytoplasm giving it granular nature Formation of follicle cells around individual
Mid vitellogenic stage	F4	The oocytes increase in size at this stage, Characteristic features of this phase was the rough granular cytoplasm due to the formation of dense yolk platelets and accumulation of lipid platelets ; small peripharel nucleoli are found. Accumulation of yolk globules proceeds intensively, and coalesces towards the center when the nucleus loses its definition.
Vitellogenic stage (Hydrated female)	F5	Very large ovary containing hydrated oocytes. Vitellogenic oocytes were characterized by the abundance of mature yolk plaelets filling the entire ooplasm. The large nucleus at the center shows a convoluted form, stained faintly , with numerous small nucleoli lying at the periphery. Lipid droplets increase in size and the single largest one at the center measure100μm .The zona radiata forms a thin non-striated oolemma.
Post-spawning female	F6	Lamellae disorganized and oocytes atretic; Extensive vascularization of the ovary.

The gonadosomatic index (GSI) was calculated as per the method described by Giese and Pearse (1974) as :

GSI = wet weight of ovary ×100/ wet weight of animal.

Total body weight and gonad weight of *E. tauvina* collected from the wild (only females) during different months were used. Being protogynous hermaphrodites, naturally transformed males were not obtained in the samples. Hormonally sex inverted males were subjected to this study.

Body weight and the gonad weight were recorded and the GSI was calculated thus:

GSI =weight of gonad / weight of fish x100.

Gonado somatic index for different length groups were determined. For this purpose, they were grouped into 25mm class interval size groups. High GSI values were observed in the size group 601 -625mm (3.25). The lowest value was observed in 401-425 mm size group (0.09). The GSI values observed for the other size groups are as follows: 426-450mm group had an average GSI of 0.44, in the length group 451-475 mm, the GSI was 0.44; the GSI observed in group of length range 476-500mm was 1.08, in the 526- 550mm size group it was 1.44. But the average GSI observed in the size group 551-575mm was 1.28. In female fishes larger than 600mm upto 650mm the average GSI observed in the present study was 3.04. The GSI values ranged from 0.05 in immature to 4.57 in mature gravid females. During development from immature stage to the pre-vitellogenic oocyte stage there is nearly four fold increase in the GSI (stage I to II , ie. 0.09 to 0.15), whereas the increase in GSI observed during transition from early vitellogenic to vitellogenic oocyte was around 7.5 times (stage II to stage III, GSI observed in this stage was 0.17 to 1.28. During transition from maturity stage IV to

stage V, this was only a three fold; a decline in GSI values was observed in spent ovaries(**Table 8.).**

Mean Gonado- Somatic Index for female *E.tauvina* ranged from 0.048 in pre-vitellogenic stage to 4.571 in mature spawning female; in sex inverted males it ranged from 0.09 to 0.131. These values were similar to those reported for other tropical serranids [eg. In *Plectropomus leopardus* for female and male fish it was 4.2 and 0.46 respectively (Frisch *et al.*, 2007,) 8 for female *E.merra*, (Lee *et al.*, 2002); 0.4 for male of *E.morio* (Johnson *et al.*,1998), and 0.2 for male of *E.merra*, (Bhandari *et al.*, 2003] and are typical for fishes with multiple group synchronous pattern of gamete development (Frisch *et al.*, 2007) . While studying the reproductive biology of *E.tauvina* from the Arabian Gulf waters, El-Sayeed and Abdel-Bary (1999) suggested a steady increase in GSI values in females from 0.45 to 4.57, from January through April.

It was observed in the present study that in *E.tauvina*, in females, peaks in Gonado - Somatic Index and mean ova diameter synchronized with lunar cycle; further, hydrated oocytes were found during successive new-moon phases. These results suggest that gonadal development in females *E.tauvina* has lunar periodicity. This is also in conformity with the actual spawning obtained in captivity in indoor tanks during the present study.

Table. 8. ***Gonadosomatic Index in female, intersex and male E.tauvina***

S.no.	Total length	Total weight	Gonad weight	Sex	GSI
1.	390	670	0.9605	F2	0.1426
2.	405	950	0.4617	F 2	0.0486
3.	450	1500	2.2350	F 2	0.149
4.	452	1345	1.3065	F3	0.0971
5.	463	800	4.4030	F3	0.538
6.	510	1650	1.201	F2	0.0727
7.	528	2050	1.7810	F3	0.0868
8.	510	2600	6.562	F3	0.252
9.	570	3200	5.0352	F4	0.1573
10.	550	2950	4.5010	F3	0.1546
11.	593	2800	4.096	F3	0.1463
12.	564	2900	10.556	F4	0.364
13.	420	1250	1.200	F2	0.0960
14.	610	3100	2.030	F3	0.0654
15.	610	2100	96.00	F5	4.571
16.	630	4626	10.00	F3	0.2161
17.	646	3500	5.4665	F3	0.1561
18.	663	4600	20.890	F6	0.4541
19.	642	4850	2.325	Intersex	0.0479
20.	653	4550	5.967	M	0.1310
21.	661	6712	14.00	F3	0.208
22.	680	5424	12.10	F3	0.2212
23.	754	6800	6.4030	M	0.0941
24.	780	5600	2.514	RE-INV	0.0449
25.	790	5550	6.384	RE-INV	0.1150
26	871	12000	8.110	RE-INV	0.0675

3.6.Discussion.

The methodology followed in the present study to define the various stages of sexual maturity in *E.tauvina* is the one that was used by Thompson and Munro(1978) in the study of maturity stages of serranids from the Caribbean sea The same methodology was also adopted by Ghorab *et al.* (1986) in studying the developmental stages of *E.chlorostigma* from the Red Sea. The developmental changes observed in the gonad of *E. tauvina* in the present study are typical of the *Epinephelus* type as elucidated by Smith (1965).

In *E.tauvina*, ovaries are of "cystovarian type" where the ovarian lumen is continuous with the oviduct. Here the gonad was observed to be comparatively of small size, the weight of the ripe gonads relative to the total weight of the fish was small as observed in the case of *E. diacanthus* (Tessy 1994). In a mature female, numerous oocytes were seen as array in the lamellae surrounding the central lumen. Inner to the ovarian wall, the inner ovarian (germinal) epithelium projects into the lumen of the ovary (ovocoel) to form fingerlike ovigerous folds or ovigerous lamellae. Oocytes belonging to different developmental stages lie in these folds in a loose connective tissue called stroma. The oocytes as they enlarge fill and extend the lamellae and the ripe oocytes liberated into the ovarian lumen move to the oviduct. All the layers are clearly seen only in immature and post-spawned ovaries, as the ovarian wall in the ripe stage become thin and highly stretched owing to the pressure exerted by large yolk laden oocytes. As in the case of mullets, (Gopalakrishnan 1993) the circular smooth muscle cells found on the wall of the ovary are involved in the release of eggs from the ovary. The right

and left lobes of the ovary of *E. tauvina* are unequal, the right lobe is bigger than the left one, as observed in the case of *E.chlorostigma* from the Red Sea. The right lobe of the ovary in the greasy grouper *E.tauvina* is larger by 10-12% than the left lobe.

The difficulty of sexing *Epinephelus spp.* on occasions other than when fully mature has been reported by many authors like Moe (1969), Tan and Tan (1974), and often necessitates histological examination of the gonads. Juveniles of *E.tauvina* measuring upto a total length of 300mm do not exhibit internal differtiation of sex. The gonads show their differtiation as fine hair like transparent strands in the earliest stage, which increase in diameter and become transluscent as they advance to stage I of the ovary. Sex can be distinguished in fishes which were about 380mm in total length and around 7months of age. A thorough knowledge of gametogenesis is necessary for evolving suitable biotechnological methods, which could be helpful in acquiring reproduction in captivity. Once the morphological development of gonad correlated with its cytological characteristics is well understood, it would be possible to predict the reproductive quality of the brooders by observing its morphological characters without further stress to the animal .

Studies on the reproductive physiology of groupers are scanty, but for a few reports from the Red Sea and Gulf coasts. As observed in the present study, Bouain and Siau (1983) found that the female gonads in three species of groupers *E.aneus, E. guaza* and *E.alexandrinus* also had a certain ripening scheme following a cyclic phenomenon, which can be divided into phases more or less arbitrarily chosen, but closely related to certain

precise cytological characteristics, such as the appearance of the nucleoli or the yolk formation. Although the vitellogenic development and spawning seasons of groupers differ from species to species, the pattern of gonadal development and the composition of oocytes are similar in all species. In the present study in *E. tauvina*, the ovary contained vitellogenic oocytes and yolk-stage oocytes in the spawning season, suggesting that several batches of oocytes may be released during the spawning period. The initiation of gonadal development and maturation is associated with cyclical environmental changes such as photoperiod and water temperature has been demonstrated in several species of groupers (Shein *et al.*, 2004).

The importance of ovarian estrogens in regulation of vitellogenesis has been demonstrated in several species of groupers. The level of E2, increased rapidly in females having gonads with oocytes in yolk vesicle stage and vitellogenic stages, suggesting that E2 has a critical role in the initiation and maintenance of vitellogenesis. A decrease in E2 levels, through inhibition by MT may stop vitellogenesis, induce oocyte degeneration and permit male development (Fostier *et al.*, 1983). Frequency of spawning was determined on the basis of the multiplicity of modes in the ova diameter frequency curves, growth of the successive egg groups and the relative number of eggs in different groups. According to Sadovy (1994), a better understanding of grouper reproductive biology would greatly facilitate stock management. Information on annual fecundity, sexual maturity, number of spawns and age and size at maturity are some of the reproductive aspects useful in management (Collins *et al.*, 1996).

Chapter IV.

Hermaphroditism, Sex inversion, Male Reproductive system and Spermatogenesis in *E. tauvina*.

4.1. Introduction

A hermaphroditic individual is one, which bears recognizable male and female tissues. In a particular species, if all or nearly all individuals possess both ovarian and testicular tissues, that species is hermaphroditic. Before sex differentiation starts in fishes, the undifferentiated gonad contains all the cell types it requires, capable of developing into either a testis or an ovary. The direction of further development and differentiation depends upon both the genetic sex chromosomes and on internal and external environmental factors (Pandian and Koteeswaran 1998, 1999a). Although the Y chromosome forms the significant factor in testicular differentiation, many other factors including autosomal genes for sex reversal and testicular feminisation can profoundly affect the fate of the presumptive gonad. There is no medullary tissue in teleost gonads; the absence of such a dual origin of gonads in teleosts is considered to be responsible for the plasticity of sex causing comparatively the more common presence of intersexuality (Brusle 1987, Brusle-Sicard *etal.*, 1992, 1994, Guraya 2000) .

The continuous presence of primordial germ cells (PGC) in immature gonads indicates that they are stem cells which constitute permanent source of germ cells, as suggested by their continuous presence in resting and active gonad (Brusle-Sicard *et al.*, 1992, 1994). In *Serranus hepatus*, the ovarian zone of the ovo-testis differentiates earlier than the male zone. Primordial germ cells migrate from female zone to colonize the testicular portion indicating the same source of two germ lines (Brusle 1987). The testicular zone forming a narrow band is placed ventral and posterior to the large ovarian zone and projects into the ovarian cavity (Reinboth 1962). No structural demarcation is seen between the ovarian and testicular zones, as the same epithelium borders the testis and the ovarian lamellae.

Three basic forms of hermaphroditism found in fishes are protogyny, in which some or all individuals function first as females and later in life function exclusively as males; protandry, in which sex transformation is from male to female and simultaneous hermaphroditism in which individuals function at the same time of life both as male and female (Sadovy and Shapiro 1987). Atz (1964) lists 13 families of teleosts that include species of these types. In protogyny, males may develop directly from the larval/juvenile stage or may develop from adult females by sex reversal. The former are called primary males and the latter secondary males. Sadovy and Shapiro (1987) and Brusle-Sicard *et al.*, (1992, 1994) concluded that the PGC have the bipotentiality to differentiate into male and female germ cells and this sex specific differentiation or change of PGC is responsible for the plasticity of sex in fish. The transformation may be accomplished in several ways, depending on the arrangement of sex tissues (Reinboth 1962,1967).

Species with one male developmental pathway, ie. all males are derived from females, are labeled monandric and those with two developmental pathways are labeled diandric. Many monandric species have bimodal size or age frequency distributions; modal size of females is less than modal size of males and modal age of females is smaller than modal age of males (Sadovy and Shapiro 1987). In protogynous fish, the transitional gonads contain clusters of spermatocytes, spermatids or spermatozoa and also should contain degenerating ovarian tissue. The gonad may also contain a central lumen, atretic follicles and small or large oocytes in early stages of atresia.

Simultaneous protogynous hermaphroditism has been confirmed in family Serranidae (Reinboth 1970, Smith 1975). Here the gonads are undelimited; male and female tissues are generally present from early stages of gonad development, though separate, lie in close proximity; oviducts and sperm ducts are separate (Brusle 1983, Brotone, 1977; Reinboth 1962). In protogynous serranids, after sex reversal, male gonads usually retain the ovarian lumen from the female stage.

At sexual transition, the oocytes degenerate, the spermatogonia proliferate and the ovary is transformed into a functional testis. Often oocytic remnants persist in the testes, which may be used in the diagnosis of female to male sex change. Thus, here the transforming gonads contain degenerating tissues of ovary and proliferating tissues of testis. Evidence of the ovarian origin of the testes are the remnants of oocytes and the ovarian lumen, can be seen in the cross-sections of testes.

But even this protogynous mode of reproduction is complicated in some species by the occurrence of large females that do not change sex and also small males that are mature at the same size as the smallest females. Even in nature an exogenous inducement of sex transformation is indicated by the sexual transition occurring over a broad range of size /(age) and also by the presence of females older than the age at which transition is completed . Smith (1967) suggested that change in sex in protogynous hermaphrodites is generally a continuous process occurring over a wide range of fish sizes / ages, hence large females may sometimes be found.

4.2. Hormone induced Sex-inversion in *E.tauvina*

The ability of sex steroids and gonadotropins to regulate sex differentiation and gametogenesis has been successfully used in fish farming for the manipulation of onset of puberty or in the induction of sex change in both gonochoristic and hermaphroditic fishes (Pandian and Sheela 1995; Piferrer,2001) . Female to male sex inversion in protogynous hermaphrodites can be achieved by treatments with androgens such as testosterone(T), 11-ketotestosterone (11-KT), and the synthetic 17∝ methyl testosterone (MT) (Starter *et al.*,2006,Yeh *et al.*, 1986,1989 and 2003).

Since keeping large male specimens of groupers in the breeding stock would be both difficult and economically unfeasible, it is necessary to have artificially sex transformed males for spawning. In order to induce artificial sex reversal and obtain an appropriate ratio of both sexes for spawning, oral administration, injection or implantation of sex steroid hormones (eg.17α-methyl testosterone and 11-

ketotestosterone) has been carried out on many species of groupers in captivity. Captive spawning of *E. tauvina* in the present study was carried out by transforming female fish to male . Sex reversal was effected by oral administration of 17∝ methyl testosterone (MT) through trash fish used as feed . The effect of MT treatment varies according to the feeding response of individual fish (Chao and Chow, 1990). The method followed by Chen *et al.*, (1977) and Chao and Chow, (1990) was adopted in the present study on sex inversion and breeding of the grouper *E.tauvina* in the laboratory and elucidated using histological and cytological criteria.

4.3. Materials and methods

Fish samples were drawn from the exprimental tanks where the treatment using the male hormone 17∝ methyl testosterone was carried out. The experiments were conducted between June 1998 and May 2000 in the facilities at the Mariculture Laboratory of the Central Marine Fisheries Research Institute at Fisheries Harbour, Cochin, using greasy grouper *E.tauvina,* collected from the wild. Fishes were maintained during the experiment period in 5 numbers of $4m^3$ circular cylindroconical FRP tanks provided with sea water recirculation facilities from the 3 numbers of in situ biofilters established in the tanks; exposed to ambient photothermal conditions and fed ad libitum with raw/frozen trash fish once a day. In order to ensure sufficient nutrition, fish were fed three times a week with squid meat or commercial seacod capsules also. Male spawners were developed in the present study through hormonal sex inversion of females. Twenty numbers of females in various stages of maturity having total length ranging from 48 - 67cm were used for sex inversion studies. The male hormone 17 α methyl testosterone,

purchased from Ms. Argent Chemicals, USA, was made into pellets using cholestrol matrix and a cellulose binder (Sherwood *et al.*, 1988). The hormone pellets implanted into trash fish were administered orally to the selected females three times a week at the normal feeding time. Hormone was administered at an average dose of 3mg/kg body weight starting from August 1998, and each fish received 12 doses per month. Diet treatment was by feeding the fishes individually. The fishes were examined periodically for the presence of milt. Before handling, the fish were starved for two days to reduce handling stress and were anaesthatised in a bath with MS222 at 100ppm dosage.

The fish were allocated randomly in 5 groups. The control fishes were also fed in the same manner except for the hormone pellet. An initial sampling was carried out at the start of the experiment . Susequent samplings were carried out on the 30^{th} day after start of hormone application, on the 40^{th} day and also on the 80^{th} day when spawning took place. Gonads were removed, weighed and fixed in 4% formaldehyde. The rest of the methodology followed is that adopted in the case of studying the female reproductive organ, which is described elsewhere in the course of this study **(Table 9 shows dosage of hormone administered) .**

Table. 9. *Dose of hormone17á methyl testosterone and corresponding sex transformation stages in E.tauvina*

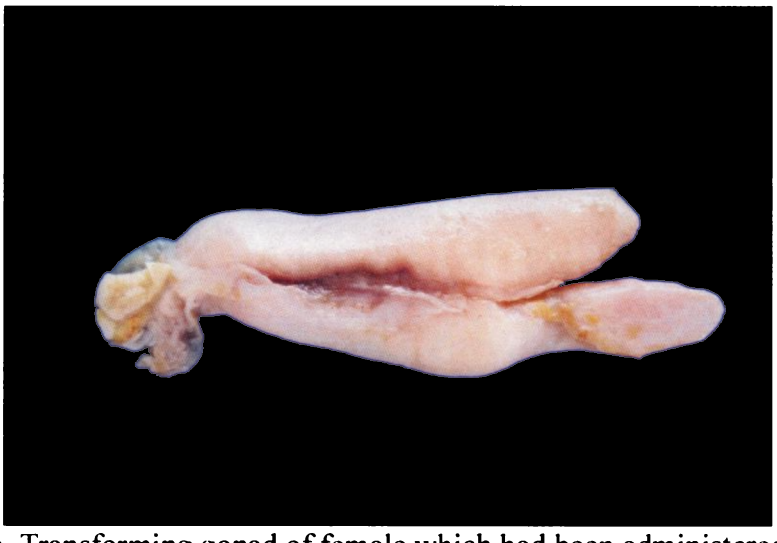
Total Dose of hormone administered	**Period of administration**	**Sex transformation, stage**	**Colour, appearance and characteristic features**
62mg (21.4mg/kg)	30 days	Transforming immature stage	The gonad appeared loose walled, elongate and compressed , pinkish ,more similar to ovary. Atresia of oocytes has taken place intermingled with spermatogonial tissue.
86mg (26.9mg/kg)	40 days	Inactive or intersexual gonad	Morphologically the gonad appeared very much similar to the testis,the two lobes flat and leaf-like; spermatogonia, apermatogonial crypts and primary spermatocytes alonwith a few degenerating oocytes and atretic bodies seen.
131.15mg (40.35mg/kg)	80 days	Mature male stage	Gonads elongated, the two lobes of equal length, flat and leaf-like.
150 mg (44.85mg/kg)	>80 days	Spermiating stage	The spermiating testes appears elongated, two lobes of equal length, flat, leaf-like with smooth dorsal surface having furrowed appearance morphologically. Seminiferous tubules packed with tailed sperms.

In individually hormone treated fish, natural spermiation was first observed after 10 weeks. The treated fishes became mature males after the administration of a mean accumulated dose of 50-55mg/kg/fish after 9-12 weeks. Sex inversion was induced in both in the smaller and the larger fish, with no clear relationship with fish size and age.

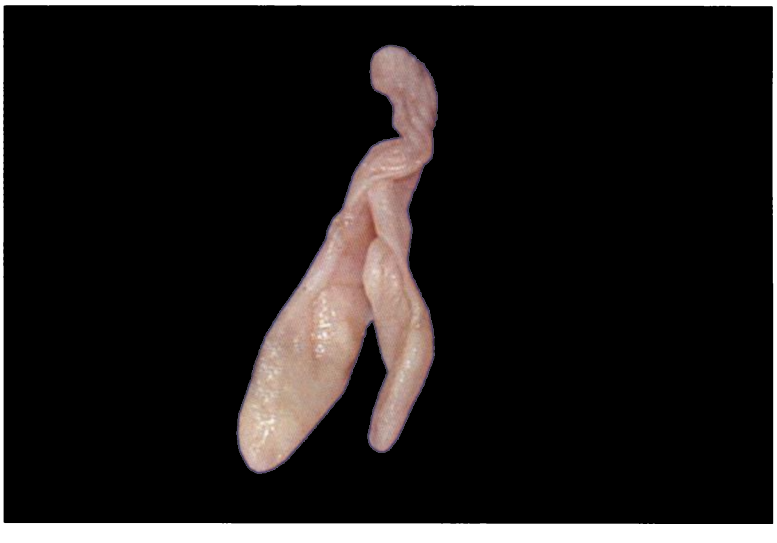
The transforming gonad : **(Plate XI a)**Gonads of mature female fishes which had been administered with the male hormone 17 α methyl testosterone for a period of 30 days. The total quantity of MT administered here was 62mg, given orally through feed at a rate of 3mg/kg body weight of the fish , three times a week. Though morphologically the gonad appeared as loose walled, more elongated and compressed structure similar to the ovary, measuring 9.2 to 9.5cm in length, histological examination revealed beginning of sex transformation. Atresia and degeneration of the perivitellogenic and chromatin nucleus stage oocytes has taken place, which were intermingled with spermatogonial tissue. This stage could be called a transforming, immature, male.

The intersexual or transitional gonad : (Plate XI b) Gonads of fishes which had been administered the male hormone testosterone for a period of 40 days . The quantity of hormone administered by this time was 86mg, which was to the tune of 26.9mg/ kg body weight /fish . At this stage also morphologically the gonad appeared very much similar to the testis, measured a total length of 6.8 - 7.6cm in fresh condition . The two lobes of the gonad, flat and leaf like, were still of subequal length as in the ovarian stage. Histologically, it can be seen that, sex change has almost taken place and it is in male

PLATE XI



a. Transforming gonad of female which had been administered 17 α methyl testoterone for 30 days.



b. Intersexual or transitional gonad which had been administered 17 α methyl testosterone for 40 days.

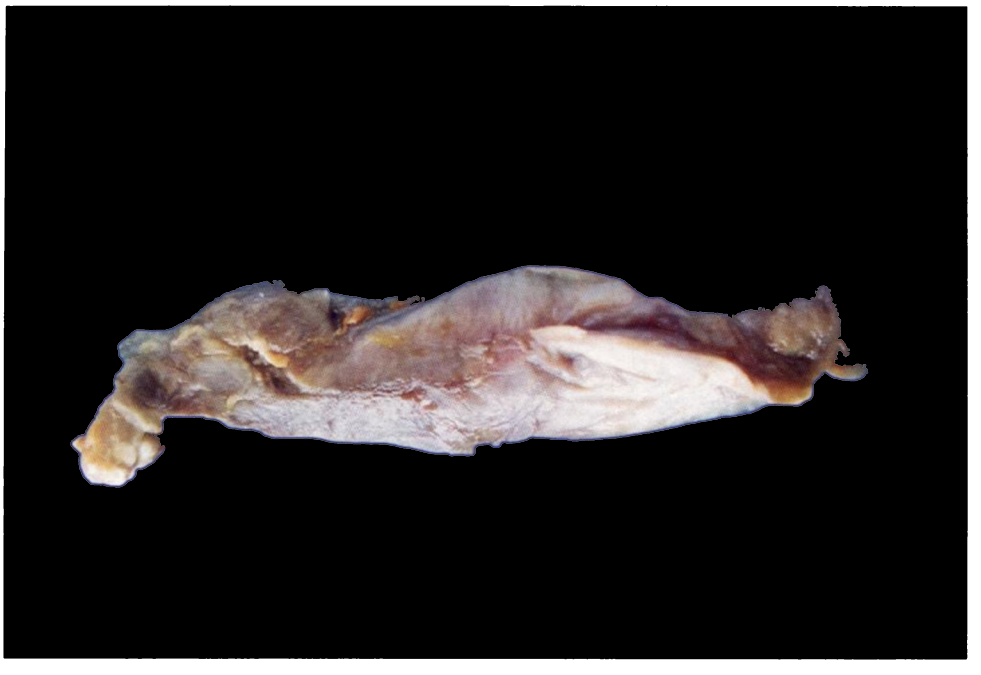
phase I. The presence of spermatogonia, spermatogonial crypts and primary spermatocytes along with a few degenerating primary oocytes or atretic bodies are noticeable. The transitional gonad at this stage can be designated as an inactive male.

Mature, spermiating gonad: **(Plate XII)** Gonads of *E.tauvina* of 56 cm total length, weighing 3.5 kg which spermiated between the 79th and 87th day after commencement of administering the male hormone, 17α methyl testosterone. The total quantity of hormone administered to this fish during this period was 131.15mg which was at a rate of 40.354mg/kg/fish. The testes further elongated and now measured 10.2- 11.4cm in length, the two lobes almost of equal length, flat and leaf like. The two lobes are elongated longitudinally, have a smooth dorsal surface, producing a furrowed appearance morphologically. Histologically the fully mature, spermiating testes, contained its seminiferous tubules packed with sperms either in multitude of crypts and as collection of tailed sperms. Collecting sinuses are dense with the collected tailed sperms and the gonad is moderately distended. Crypts of spermatogonia and spermatocytes were not observed in this stage of fully ripe testis.

4.4. Male Reproductive System in *E.tauvina.*

Among serranids males may develop directly from the larval/juvenile stage or may develop from adult females by sex reversal. The former are called primary males and the latter secondary males.

PLATE XII

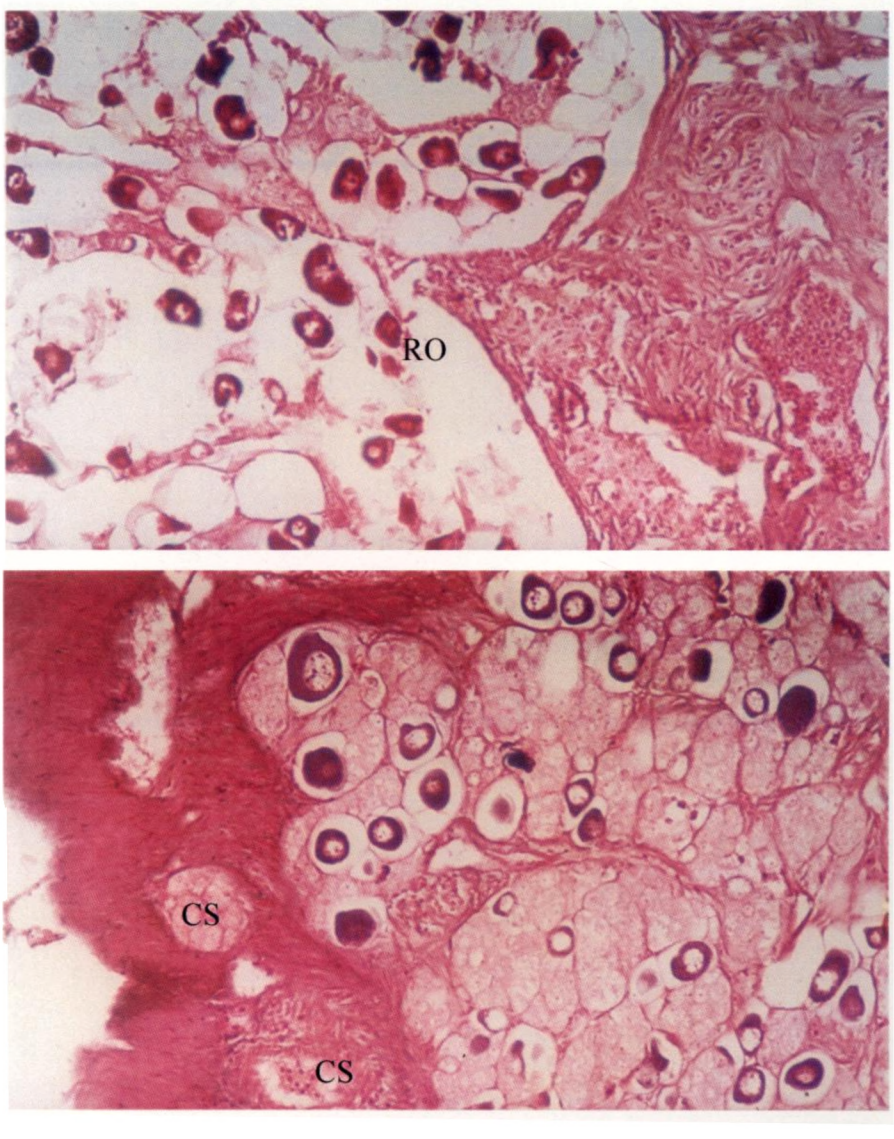


Mature gonad of a fully sex inverted male

The male tissue of *E.tauvina* is of the acinus type in which the sperms are formed in small crypts which are surrounded by connective tissue membranes and all the cells within a given crypt are at the same stage of meiosis. The persistence of oocytes arrested in the stage of primary growth phase was frequently observed in fully formed testes, corroborating the findings of Smith (1965) and Brusle & Brusle (1975b). Similar to the observations in the present study, Hastings (1981)has described three modes of resorption of ovarian tissue in the transforming gonads of *Hemanthias vivanus* including fragmentation of pre-vitellogenic oocytes, and breakdown of unovulated eggs. Saidapur (1978) states that the invasion and breakdown of mature eggs is similar to the pattern typically associated with resorption of unshed eggs.

The testes are a pair of flat, elongated structures, much smaller than the ovary, lying ventral to the kidney and dorsal to the alimentary canal, between the posterior part of the swim bladder and the ventrolateral wall of the abdominal cavity, connected by mesenteries. Thick peritoneal layer covers them externally. In transverse section, the testis is wedge shaped, the outer, more dorsal edge forming the apex of the wedge. The dorsal surface, which is ad pressed to the swim bladder, is smooth, whereas the ventral surface is convoluted, divided into leaf like lobes. These lobes are elongated longitudinally, giving a furrowed appearance. The immature testis is transparent, but with maturity its colour becomes creamy white. During breeding season, when the testes becomes matured, it is richly supplied with blood capillaries, the tunica albuginea becomes thin and it is fully packed with seminiferous tubules . The transitional gonads, depending on the stage of transformation, morphologically appear elongated, thin,

PLATE XIII



Photomicrograph of sections of transforming gonad. Male tissue proliferating from the peripheral region (RO) regressing oocytes (CS) cysts of sperm tissue X200

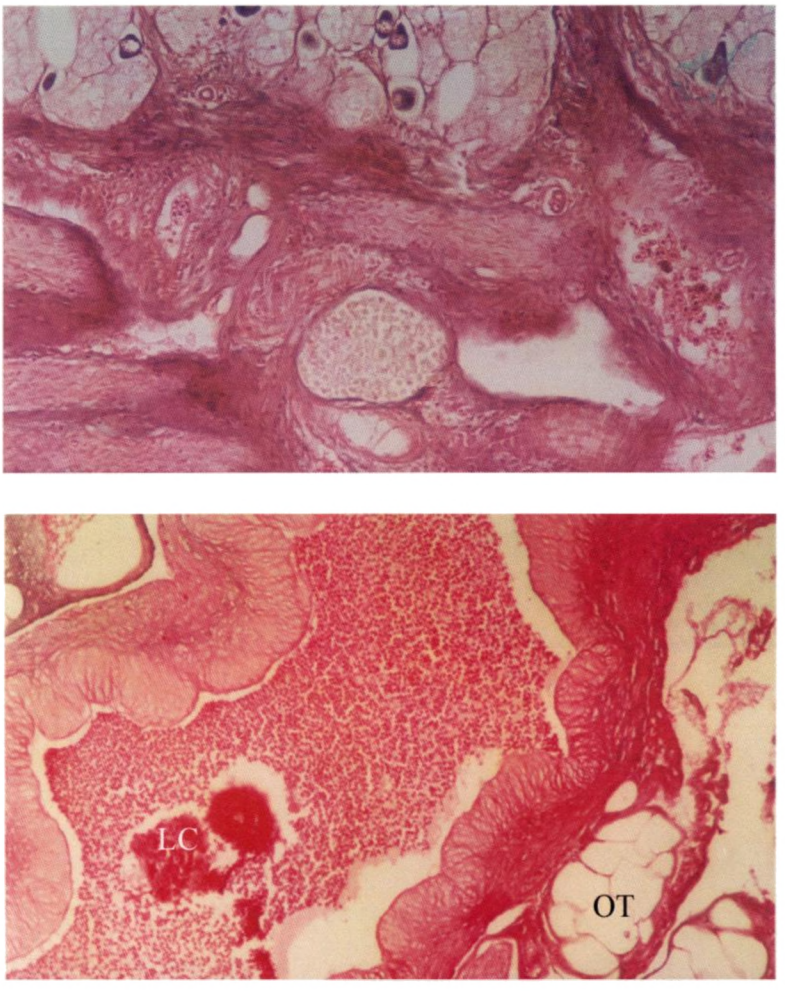
thread-like, delicate and translucent with convoluted ventral surface . The colour of the mature testes is pinkish red due to enhanced blood circulation.

Based on morphological and histological investigations, the maturity stages could be classified into three stages viz.immature, inactive, mature.

Stage I **Transforming, immature, male: (PlateXIa, XIII a&b)** Morphologically, the gonad appeared as loose walled, more elongated and compressed structure measuring 9.5cm in length, resembling more like the ovary. The gonads retained the ovarian structure with a lumen and the male tissue was proliferating mainly at the periphery of the ovarian lamellae around the ovarian cavity. These also showed leftover primary oocytes in various stages of apoptosis and crypts of sperm tissue occurred in pockets among the degenerating oocytes. Yellow brown bodies were also seen. In some of the atretic previtellogenic oocytes, outer cytoplasm was less basophilic and had split from the central mass of cytoplasm; the nucleus also appeared atretic and disappeared early in the resorption process. Zona radiata deteriorated and lost its striations; granulosa cells became hypertrophied, yolk globules ruptured and oil droplets fused. This stage could be called a transforming, immature, male. Formation of sperm duct begins in the transitional gonad along with simultaneous degeneration of ovarian follicles.

Stage II **Inactive male: (Plate XI b,XIV a & b)** Morphologically the gonad appeared similar to the ovary, but smaller than the ovary of untreated fish; measured a total length of 6.8cm in fresh condition . The dorsal blood vessel with numerous capillaries carried blood upto the lamellae. The two lobes of the gonad, flat and leaf like, were still of

PLATE XIV

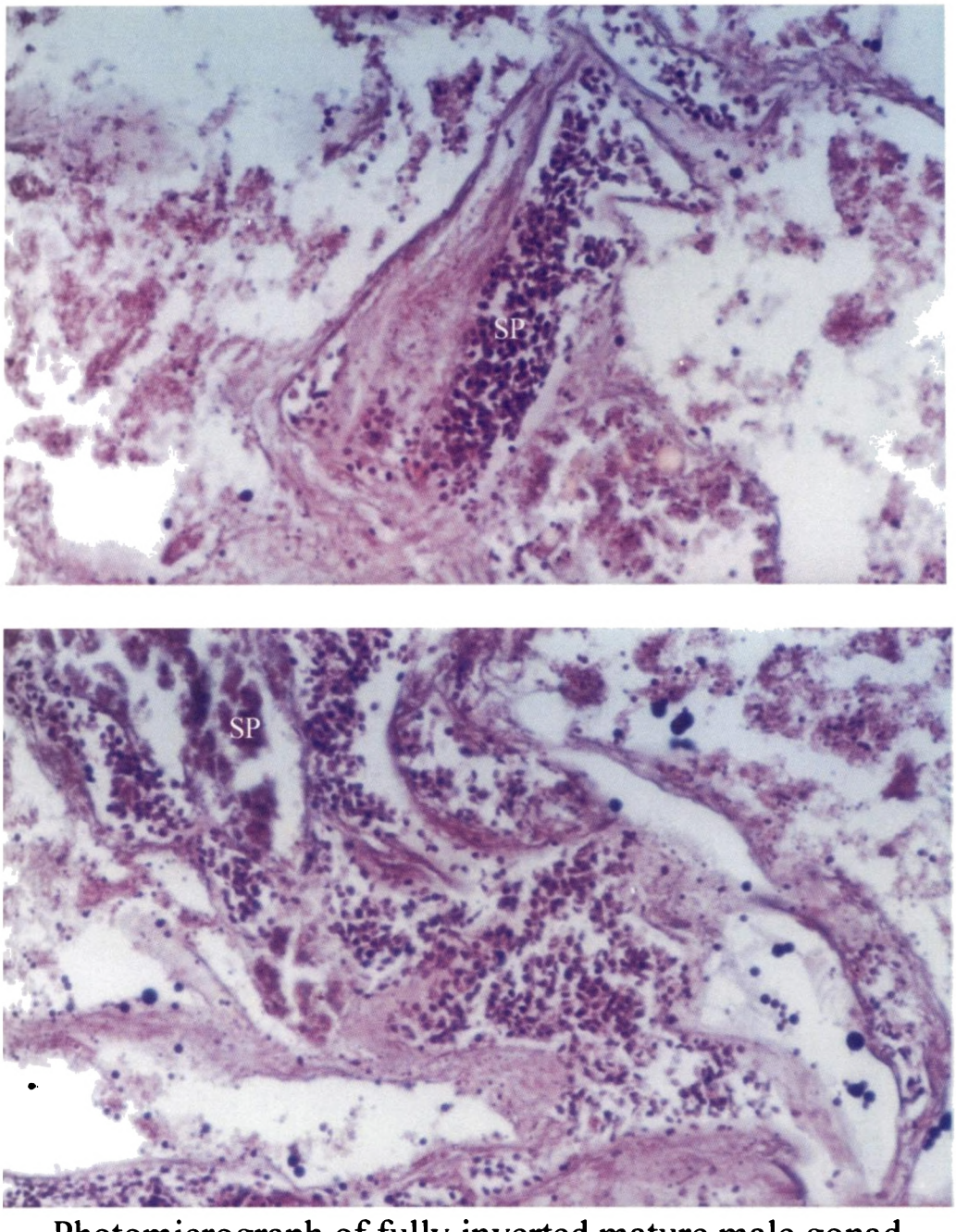


Light microscopic section of gonad of sex transforming male showing sperm cysts and degenerating ovarian tissue (OT), leydig cells (LC) X200

subequal length, as in the ovarian stage. Histologically, it could be seen that, the gonads still retained the ovarian cavity. Lamellar structures were completely converted into lobular structure of the testis with spermatogenic tissue in small crypts on the periphery ; sex change has almost taken place and it is in male phase I. The lobules were filled with cysts containing spermatocytes, spermatids or the sperm mother cells; spermatogonial crypts and primary spermatocytes along with a few degenerating primary oocytes or atretic bodies. A distinct type of cells similar in size to blood vessels and characterized by ovoid nucleus and pink staining cytoplasm were present within the lamellae similar to those seen in sex changing *E.rivulatue* (Mackie 2006) . These were thought to be Leydig cells which occurred only at the time of sex change. This stage can be designated an inactive male.

Stage III Mature male: (Plate XII, XVI a,b) The ripe testes measured @10.5cm in length, the two lobes almost of equal length. The two lobes were ribbon like, elongated longitudinally, have a smooth dorsal surface with irregular ridge, white and oval in shape. Testis are covered with connective tissue by which it is connected to the peritoneum, filled with foldings of seminiferous tubules. The dorsal blood vessel was large and other blood vessels were also present within the thick muscular tunica. Histologically this fully mature, spermiating testis maintained the ovarian cavity, its seminiferous tubules were packed with sperms either in multitude of crypts and as collection of tailed sperms. Spermiogenesis was under way and some crypts of spermatozoa had ruptured releasing their contents into the lobular lumen. The sperm ducts and the peripheral sinuses were filled with sperm. Collecting sinuses were dense with the collected tailed sperms and the

PLATE XVI



Photomicrograph of fully inverted mature male gonad showing spermatozoa or sperm (SP) abundant in the lumen X400

gonad is moderately distended. Crypts of spermatogonia and spermatocytes were not observed in this stage of fully ripe testis.

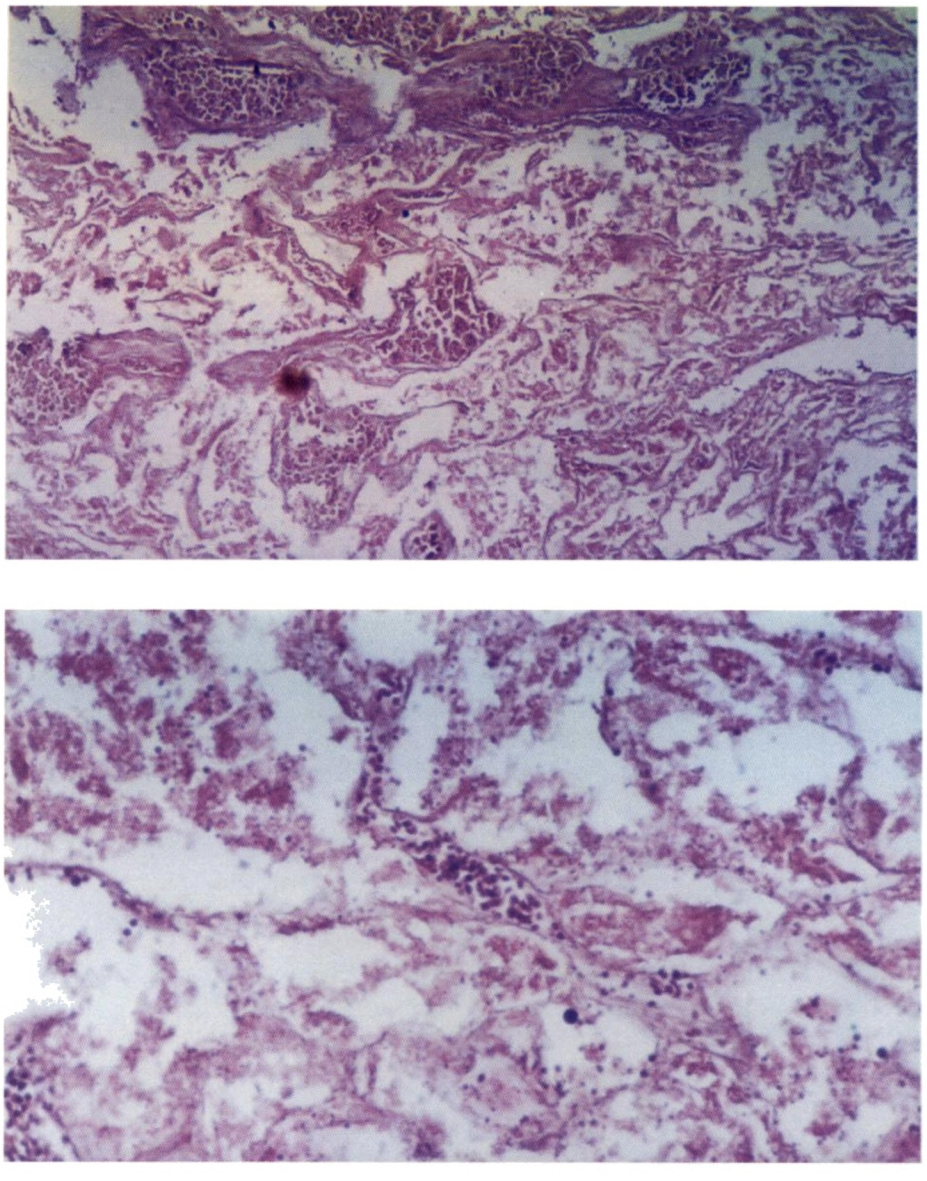
The testes of post-spawning males were large and flaccid, producing a furrowed appearance morphologically and contained numerous empty lumina, and also contain proliferations of gonial cells. Sperm sinuses were discernible which contained very little or no sperm **(Plate XVII).** Sperm could not be obtained using abdominal pressure, but histological appearane of the testes was indicative of the completion of spermiation period.

During spawning activity most crypts are filled with mature sperms and only a few spermatogonia. The connective tissue membrane surrounding the crypts breakdown when sperms reach maturity. The persistence of oocytes arrested in the stage of primary growth phase was frequently observed in fully formed testes, corroborating the findings of Smith (1965) and Brusle & Brusle (1975b). Similar to the observations in the present study, Hastings (1981)has described three modes of resorption of ovarian tissue in the transforming gonads of *Hemanthias vivanus* including fragmentation of pre-vitellogenic oocytes, and breakdown of unovulated eggs. Saidapur (1978) states that the invasion and breakdown of mature eggs is similar to the pattern typically associated with resorption of unshed eggs. As the gonad was cleared of ovarian tissue, the rate of spermatogenesis increased and the lamellae soon became dominated by spermtocytes and connective tissue. Putative Leydig cells proliferated when sperm tissue was well under way and were more abundant within the gonads of immature males (Mackie

Table. 10. ***Developmental stages and Histological characteristics of gonad of male in E.tauvina***

Sl.No.	Developmental stages	Symbol	Defining characteristics
1.	Transitional (Immature stage)	T	Gonad resembles ovary; contains ovarian lamellae; vitellogenic oocytes or pre-vitellogenic oocytes and testicular tissue present in crypts.
2.		M1	Spermatocytes and spermatids present, but spermatozoa absent. Atretic bodies often present.
3.	Inactive male (resting male)	M2	Gonad becomes smaller, dominated by testicular lobules. Spermatids present, sperm sinuses undeveloped. Pre-vitellogenic oocytes may remain.
4.	Mature male. (Ripe-running male)	M3	The ripe testes measured >10.5cm in length, the two lobes almost of equal length. The two lobes are ribbon like, elongated longitudinally, have a smooth dorsal surface with irregular ridge, white and oval in shape. Crypts of spermatozoa are abundant, sperm sinuses well developed and filled with spermatozoa.
5.	Post spawning male		Testes large and flaccid, producing furrowed appearance morphologically, Sperm sinuses contained very little or no sperm; contained proliferation of gonial cells.

PLATE XVII



Photomicrograph of fully inverted spent male gonad showing numerous empty sperm sinuses X200

2006). Yeung *et al.*, (1985) determined that during sex change, Leydig cells were responsible for a change in steroid metabolism towards the production of androstenedione and 11-ketotestosterone. Production of 11-ketotestosterone also increased in parallel to an increase in Leydig cells and spermatogonia during sex change in *T.duperrey*. It is likely that these distinctive cells observed only within the transitional gonads and immature testes were also involved in steroid metabolism(Nakamura *et al.*, 1989).

4.5 Spermatogenesis

Spermatogonia or the sperm mother cells, through a process of cytological changes metamorphose into potentially functional gametes of spermatozoa or sperm. This process or sequence of transformation of the primordial germ cells to mature spermatozoa, is called spermatogenesis. The different stages of spermatogenesis are distinguished on the basis of the size of the cells, nuclear characteristics and the cytoplasmic morphology. Spermatogenesis takes place in the seminiferous lobules that fill the lamellae and the mature sperms were released into the collecting ducts. In the 'acinus type' testicular tissue the sperms were formed in small crypts (acini) and all cells within each crypt were at the same stage of meiosis (Smith, 1965). These crypts were surrounded by connective tissue membranes that breakdown when sperms reach maturity and groups of sperm cells were isolated during spermatogenesis. The sperm sinuses and ducts were developed only during the male phase in *E. tauvina*. Pre-spawning individuals have crypts with primary spermatocytes, some crypts containing secondary spermatocytes,

many crypts with spermatogonia and some containing tailed sperms . Crypts were filled with mature sperms at peak spawning , after which most crypts were empty.

Stage I: (Plate XIII a ,b)

Spermatogonia arise as proliferations from the primordial germ cells which can be identified in *E.tauvina* during sex inversion as gathered in nests at the onset of the transformational process and also during spermatogonial proliferation. The primordial germ cells were more numerous in sex transforming fish than in males or females and lie distributed in the connective tissue stroma of the testis. In this stage, the spermatogonia were found in large numbers along the peripheral walls of lamellae. Numerous previtellogenic and regressing oocytes still remained. Spermatogonia are ovoid cells with regular and smooth cell membrane and have a pale cytoplasm and a large central nucleus having regular nuclear membrane. The spermatogonia after a period of growth multiply and form cysts of primary spermatocytes held together by the cytoplasmic processes of the Sertolli cells. As proliferation of cells in the cysts occur, the lumen of the lobules increases. All spermatogonia do not develop into cysts but may remain in the dormant condition.

Stage II: (PlateXIV a, b)

The spermatogonia, which are abundant, develop into primary spermatocytes through cell division and reduction of cytoplasm. A single spermatogonium gives rise to cysts of primary spermatocytes **(XIV b)** much smaller than the spermatogonia. Primary spermatcytes, 5μm in diameter contain a central nucleus 2.5-3.5μm, do not have visible

nuclear membrane, but with patches of densely staining chromatin material. Each primary spermatocyte in the cyst undergoes first meiotic division (reduction division) and give rise two secondary spermatocytes.

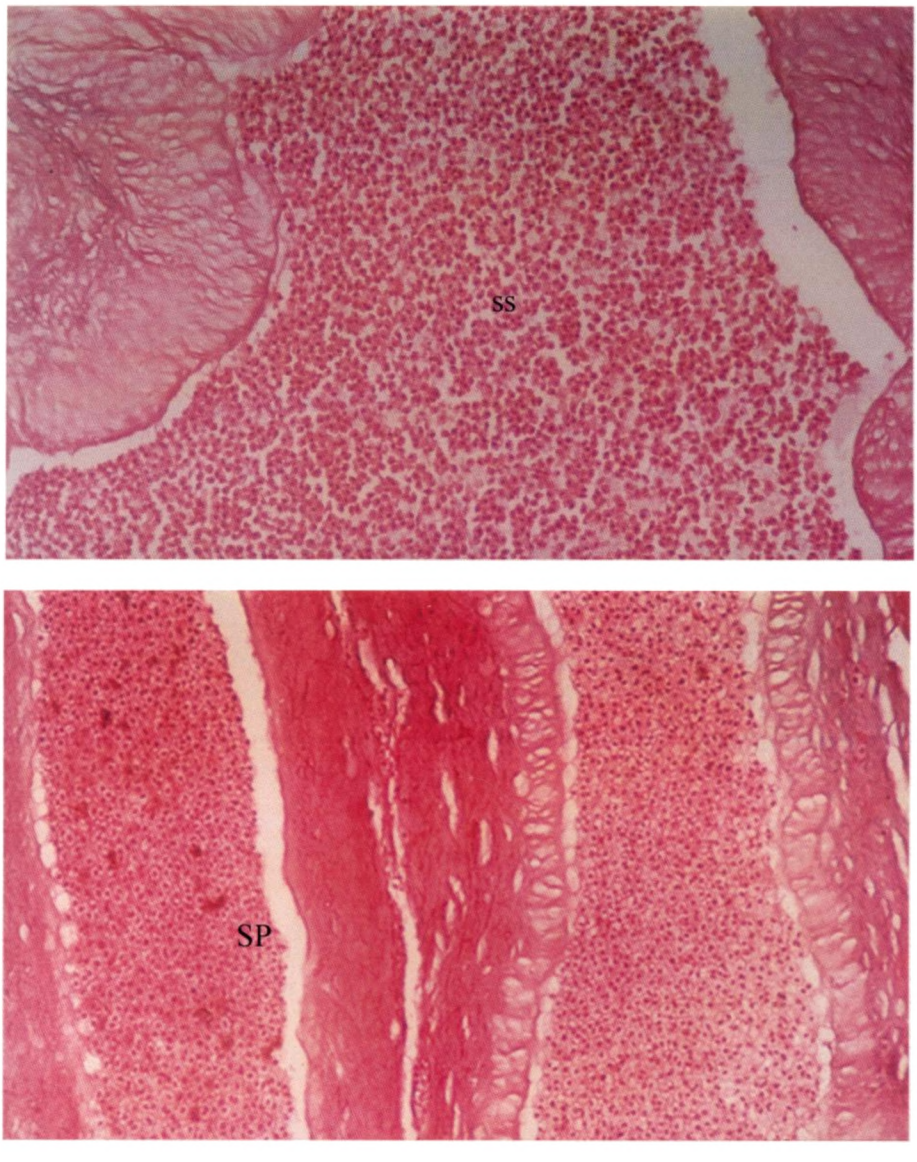
Stage III: (Plate XV a)

Groups of secondary spermatocytes, were distinguishable from the primary spermatocytes by their small size (3μm) and homogenously staining nuclei; formed through first meiotic cell division of the primary spermatocytes and subsequent concentration of nuclear material. Nuclear division take place followed by cytoplasmic divisions. The chromatin matter is granular and the nucleolus is no longer visible. Large number of primary and secondary spermatocytes are formed.

Stage IV: (Plate XV b)

Secondary spermatocytes were found in the later stages of maturation and occur in large numbers in expanded crypts. Each secondary spermatcyte undergoes a second meiotic division and gives rise to two spermatids. Spermatids smaller than (1-1.5μm) spermatcytes are seen. Secondary spermatocytes and spermatids are seen in large numbers. Early stage spermatids are characterised by the appearance of intracellular lumen within the cysts, which may be due to the shrinkage of cells. The spermatids undergo cellular organization coupled with the formation of the tail and transform into the spermatozoa. The chromatin matter undergoes condensation and becomes highly electron dense. The spermatids are 1.5 –2 μm in diameter and have intensely staining nucelei occupying most of the cell. The testes becomes pink in colour.

PLATE XV



Photomicrograph of secton of gonad showing secondary spermatocytes (SS) and spermatids(SP) X200

Stage V: (Plate XVI)

Spermatids metamorphose into motile and potentially functional gametes called spermatozoa, spermia or sperm, seen abundantly in lumen of the testis; this process of spermatid metamorphosis to motile, tailed sperms is often called 'spermiogenesis'. Here there is no cell division, but only cellular re-organisation takes place. Histologically this fully mature, spermiating testis, contains its seminiferous tubules packed with sperms either in multitude of crypts and as collection of tailed sperms not interconnected by cytoplasmic bridges. Each spermatozoa is essentially composed of three regions viz. the head, neck and flagellum. Collecting sinuses were dense with the collected tailed sperms and the gonad was moderately distended. Crypts of spermatogonia and spermatocytes were not observed in this stage of fully ripe testis. The testes has become elongated and enlarged in this stage.

After spawning the testes contained numerous empty sperm sinuses which were discernible but they contained little or no sperm.(**Plate XVII)** The internal stucture which was disorganized also showed proliferations of gonial cells. The testes decrease in volume and size.

4.6 Discussion

Groupers of the genus *Epinephelus* primarily display protogynous hermaphroditism. In *Epinephelus tauvina*; inversion of sex is brought about by development of the testicular region and regression of the ovarian part, though external

distinguishing characters are not visible in either stage. As observed by Sadovy and Shapiro (1987) and Brule *et al.*, (2003), the main criteria for identifying protogyny in hermaphroditic fishes were clearly observed during the present study, viz. membrane-lined central cavities in in the testes, sperm sinuses in the gonadal wall and transitional individuals. The gonads contain clusters of spermatocytes, spermatids or spermatozoa with degenerating ovarian tissue; the seminiferous nests could be precocious spermatocytes and sperm cysts present in functional ovaries. Similar to this scattered sperm cysts were observed in immature and mature ovaries in *E. fulvus, E.crenuatus* and *E.flavolimbatus* (Smith 1964, 1965; Bullock *et al.*, 1996).

Like other species of the genus Epinephelus (Sadovy and Shapiro 1987, Smith 1975), in *E.tauvina* also the male and female tissues were not separated by connective tissue; but within that region the male and female elements lie side by side, without an intervening connective tissue wall . In the greasy grouper, in the present study mature oocytes and spermatozoa were never seen at the same time in the gonad, showing that it can be considered as a sequential hermaphrodite.

Various authors have reported that groupers change sex or transform into males when they grow bigger and older, between ages of 10 and 17 years.(Tan and Tan ,1974, Brusle 1985, Chauvet 1988). (Although combinations of sex genes are determined at the time of fertilization, sex is actually determined by sex-gene-controlled sex inducers at a certain critical period of development.) One of the difficulties in defining the time of sex change from the occurrence of transitionals in a population is their low percentage in

collections. (Shapiro 1987, Johnson *et al.*, 1998). Histological studies conducted by Tan and Tan (1974), showed that the biological minimum size of *E.tauvina* from the South China Sea, showed that fishes in the size range of 450-500mm were females while male fish with ripe testes were above 740mm and more than 11kg body weight; transitional gonads containing male and female gonadal tissues occurred in fish of 660-720mm. Genetic, exogenously applied sex hormones and various environmental factors are observed to cause change of sex in fish (Guraya 1994, 2000, Pandian and Koteeswaran 1999a, Pandian *et al.*, 1999). But the precise nature of genetic and biochemical mechanisms involved remain to be determined at the cellular and molecular level. Lee *et al.*, (1995) have investigated the steroidogenic potential of gonadal tissue of the protogynous grouper *E.tauvina* before and after sex inversion using 17α methyl testosterone.

Sex reversal is a very common practice in aquaculture; the main goal was the production of monosex population suitable for breeding (Hunter and Donaldson 1983). Unstable sex differentiation in teleosts allows various sex hormones to be used to influence sex expression in many species of fish (Pandian and Sheela 1995). Sex reversal has also been described for several species of groupers such as, *E.fario* (Kuo *et al.*, 1988), *E. suillus* (Tan Fermin *et al.*, 1994) *E.marginatus* (Glamuzina *et al.*, 1998,) *E. coioides* (Yeh *et al.*, 2003) etc. besides *E. tauvina* (Chen *et al.*, 1977 and Chao and Chow, 1990).

To date, the male sex hormone 17α methyl testosterone (MT) has been commonly used in varying doses for sex-inversion. Testosterone appears to have an important role in spermatogenesis in males and transitionals. In protogynous hermaphrodites, mainly members of genus *Epinephelus*, ability of MT to accomplish female- to-male sex change has been demonstrated well (Marino *et al.*, 2000, Lee *et al* ., 2002, Yeh *et al.*, 2003b). Oral administration of 145mg /MT/kg/BW for one year (Chen *et al.*, 1977) and 120 mg/MT/kg BW for *E. tauvina* (Chao and Chow ,1990) 70 mg/MT/kg BW, for *E . fario* and 104 mg/MT/kg BW in *E.salmonoides* (Kuo *et al.*, 1988) has successfully induced sex change in these groupers . Injection of 30mg/MT/kg BW (0.8-1.5 kgBW, six biweekly injections) induced male grouper in *E.suillus* (Tan Fermin et *al.*, 1994). Implantation of MT (0.5 mg/MT/kg) in silastic capsule for 4 months resulted in functional males in *E. tauvina* (Chao and Lim 1991). The effective dose also depends upon the initial ovarian stage when the hormone application was given (Yeh *et al.*, 2003). The optimal dose and the time limit for sex change vary among species of grouper.

In the present study, regardless of the initial stage of ovarian development, degeneration of ovarian tissue progressed to the transitional stage of sex reversal when the accumulated dose of MT reached a level of 20mg/kg body weight of the fish which is similar to that observed in *E. fario* (Kuo *et al.*, 1988). Again, in the present study spermiation was possible when the MT dose reached a level of 40.4mg/kg body weight, resulting a fertilization rate of 80-95% . By adding 1mg kg^{1} dose of MT to food resulted in sex reversal to male in 3 year old *E.tauvina*; the female fish became fully functional male when the accumulated quantities of MT reached the level of 145mg and the

resulting sperm successfully fertilizing the eggs (Chen *et al.*, (1977). In *E. fario*, 2 year old fish were successfully sex reversed by adding 0.5mg and 1.0mg kg^{1} MT to food over a 5 month period. (Kuo *et al* .,1988).

Transitional individuals are relatively uncommon in field collections; this low incidence could be due to many facts like, (a) sex reversal occurs seasonally in some species, probably at the end of spawning season (Reinboth 1962, Chan and Phillips,1967, Brusle and Brusle 1975, Jones 1980a) or (b) these are capable of changing sex throughout the year and transitionals found year round (Chen *et al.*, 1980, Ross 1982 , Shapiro *et al.*,1993); (c) depending on the seasonality of sex change, collection time may not coincide and d) when completion time for sex change is short, sporadic sampling may miss transitionals. Another reason could be, e) in some species, only very few individuals may change sex (Warner 1982). The posterior-anterior gradient in the distribution of male and female tissue in the gonad may also lead to incorrect assessment of relative proportion of transitional gonads in the population. Therefore, experimental induction of sex change may be the only method capable of establishing that the species is sequential hermaphrodite (Cole 1983).

The size/age range of females overlaps very little with that of males and with females changing sex upon attaining some particular size or age. Many authors have studied protogynous hermaphroditism in *Epinephelus* species by studying the length frequency distribution (Tan and Tan 1974, Smith 1959, Thompson and Munro 1978). But some females in species which may not be sexually dimorphic for size (*Lethrinus*

nebulosus, Young and Martin,1982) may continue to grow throughout their life without changing sex, either because they are genetically incapable of doing so or because environmental conditions controlling sex change have not impinged upon them prior to death, and attain similar maximum size as males (Smith1962, Brusle and Brusle 1975,Waltz *et al.*, 1982).

Chan *et al.*, (1972) and Reinboth (1962), biopsied the gonads of individuals serially throughout sex change and revealed successive alterations in gonadal structure and contents. In sequential hermaphrodites as in protogynous serranids, sex change is usually accompanied by degeneration of the germinal tissue of one sex and development of germinal tissue of the other. Concomitant with germinal changes during and following sex reversal are structural changes of the gonad and its ducts (Moe 1969, Shapiro 1981, Young and Martin 1982, Cole 1983). The possible advantages of sequential hermaphroditism in fish has been discussed by several workers like Moe (1969), Warner (1975) and Charnov (1982). Using the "size advantage model', Warner (1975) suggested that the fish, by spending only part of the mature life span as female and part as male, may yield a higher expected reproductive potential when compared with non-hermaphrodites. Moe (1969) also suggested that sequential hermaphroditism can be used as a population control. For groupers, the causes of sex change must be analysed to estimate how the fishing activity could influence the population's reproductive output and to determine the effects of a protogynic hermaphroditic reproductive strategy on management measures (Shapiro 1987).

Chapter V.

Breeding Biology of grouper *E. tauvina* .

5.1. Introduction.

Groupers have recently become one of the most important aquaculture and trade commodities in the Asia-Pacific region. It is also an important fish in the livelihoods of small and large-scale coastal fish farmers. The intensified trade in live groupers resulted from a number of recent developments: increased consumption and high cultural and social preferance for this fish; the growing live seafood markets and restaurants in many of the South east Asian countries and intensified aquaculture due to high economic returns. Groupers are now considered a high-value species with a high potential for contributing to the economic development of many of these countries. Hong Kong, China, China PR and Singapore are the main markets for live grouper and the main suppliers are Indonesia, Phillippines, Malayasia, Thailand, Vietnam and Australia . Owing to the increasing demand, high market value, fast growth rate and disease resistance, there is strong interest in grouper aquaculture throught the world. At the same time, in many tropical and temperate areas, overfishing and environmental degradation are depleting wild grouper populations (Sadovy, 1993) and studies on the biology of groupers are in progress in order to provide a basis for fisheries management.

However, growth and development of grouper farming industry has been constrained by an inadequate supply of fish juveniles for stocking (Chao and Lim 1991) The existing supply of wild-caught juveniles cannot meet the demand of the expanding grouper culture industry. The development of this industry therefore is reliant upon the successful hatchery production of grouper juveniles.

In view of the unstable and declining supply of wild seed sources, techniques on artificial breeding and spawning are continuously studied and improved to provide alternate source of seeds. An important aspect is broodstock development and conditioning which involve promoting sexual maturation and enhancing broodstock quality to ensure better quality of eggs and sperm.

Another area of research is focused on methods of sex reversal. Groupers are protogynous hermaphrodites, which mean fishes mature initially as females but later on transform into males. Therefore, the commonly found males are bigger, older than females, and more aggressive which make it difficult to handle them for breeding . Hormonal induction, by the use of 17 –alpha methyl testosterone or leutinizing hormone-releasing hormone analogue (LHRHa), either by oral administration or by implantation are undertaken to produce smaller and more docile males. Social control is another means of sex inversion, by manipulating several factors such as social condition and environment, stocking density, sex ratio etc. within the holding tanks.

Spawning can be induced using a variety of hormones such as human chorionic gonadotropin, leutinizing hormone-releasing hormone analog, and pituitary glands of several fishes, singly or in combination. Manipulation of environmental conditions such as increase in temperature, water flow or water exchange are employed. Lunar cycle influences spawning and should be taken into consideration in both natural and artificial spawning methods. Artificial spawning can be done by stripping the sexual products and mixing them eithr by the dry or wet method. Or after hormonal induction, broodstock can be left to spawn naturally by providing suitable conditions

Although methods of controlled breeding and larviculture of groupers have been developed since late 1970s grouper aquaculture is still far from full commercialization owing to the shortage of fingerlings from the wild and lack of reliable technology for hatchery production. (Lim 1993; Kuo 1995 ; Watanabe *et al.*,1995). Natural or induced spawning in groupers was reported in *E. tauvina* (Chen *et al.*, 1977, Hussain and Higuchi, 1980), *E.malabaricus* (Ruangpunit *et al.*, 1986), *E.salmoides* (Kungvankij *et al.*, 1986), *E.fuscoguttatus* (Lim *et al* 1990), *E.suillus* (Toledo *et al.*,1993), *E.polyphekadion* and *Cromileptis altivelis* (Sugama and Ikenoue 1999.)

Groupers are highly fecund fishes. A mature female *E.suillus* weighing 5.3kg paired with two permiating males of 6kg and 6.5kg spawned successively five to ten times a month from July to October 1990 . Six mature females (3.5kg to 5.0 kg weight) and four mature males (weighing 7 kg to 12kg) maintained in 50tonne capacity tank spawned successively five to seventeen days a month for almost a year (Toledo 2002).

The number of eggs collected varied from 0.5 to 15.9million every month from spawning in tanks and from floating cages this varied from 2.3 to 3.9million. The mean fertilization rate varied from 67 to 88% in tank spawning and 2 to 81% incages; the mean hatching rate similarly was 72 to 89% for those spawned in tanks and it was 29 to 68% from cages. High variations in the quality and quantity of spawns may be related to fluctuations in environmental conditions and inconsistent nutritional quality of feed given to the broodstock.

5.2 Broodstock Development.

The primary requisite in any successful hatchery operation is the availability of good number of healthy male and female broodstock of the candidate species. Attempts to breed groupers in captivity started about four decades ago. Ukawa *et al.,(*1966) described the successful fertilization and embryonic development of the red grouper *E. akaara.* Fueled by the high market value of live groupers and the inconsistent supply of juveniles from the wild , research on broodstock development and seed production of grouper has been intensified since the 1980s.

There are two sources of broodstock; the wild caught adults and those reared in ponds, tanks or cages. For grouper fry production, collection of broodstock is the first bottleneck because mature fish are less available in captivity. It is advantageous to use pond or cage reared broodstock as they are already used to culture conditions, are thus easier to develop into broodstock. Pond reared groupers have better survival rate and breeding performance than wild-caught broodstock (Liao *et al.*, 2001) The difficulties

experienced in keeping large groupers alive after capture, in the offshore waters have made it necessary to develop broodstocks by rearing from juveniles. This method also enables the history and biodata of broodstocks to be traced. The sub-adults of groupers can be grown in production cages; measurements of standard length and body weight recorded at intervals. On attaining a mean individual weight of 1kg, these can be restocked in several brooder cages of 50mm mesh size at a density of 10kg/m^2. Broodstock can also be developed by rearing the juveniles in onshore tanks or ponds in seawater of suitable salinity.

To ensure good quality eggs, dietary lipid composition in broodstock feed is important. The quality and quantity of ώ-3 HUFAs contained in fish feeds are found to influence development of gonads and quality of eggs (Navas *et al.*, 1998; Sargant *et al.*, 1999).

An important aspect is broodstock conditioning which centers on promoting sexual maturation and enhancing broodstock quality to ensure better quality of eggs and sperm. During conditioning, optimum temperature, salinity, dissolved oxygen and water exchange should be maintained. Broodstocks should be well provided with feed, oftentimes needing a supplementation of vitamins, minerals, and essential fatty acids. A matured female is characterized by a fullness of the belly, protrusion of urogenital section, fully yolked oocytes and sometimes by the changes in the normal color pattern. While in a matured male, milt flows when the abdomen is pressed.

Groupers are protogynous hermaprodites, which means that they mature initially as females but later on transform into males. Therefore, the commonly found males are bigger, older (than the females), and more aggressive which makes them difficult to handle during the breeding process. Hormonal induction, such as the use of 17-alpha methyl-testosterone or luteinizing hormone-relasing hormone analog (LHRHa), either by oral administration, injection, or implantation are undertaken to produce smaller and more docile males. Social control is another means of sex inversion. Sex change may be induced by manipulating several factors such as social condition and environment, stocking density, sex ratio, and capacity of holding tanks.

The nutrition of the broodstock is an important factor for gonad development and fecundity to ensure good quality spawn (Watanabe, 1985). Moreover, spawning and egg quality are affected by the quality and quantity of feed. Toledo *et. al.*, (1993) mentioned that nutritional deficiency could be one of the reasons for having inconsistent quality of spawns of *E. coioides*. To improve the the gonadal quality, the brooders were fed trash fish enriched with commercial fatty acid boosters, Vitamins A,E etc.

Natural or induced spawning in groupers was reported in *E. tauvina* (Chen *et al.*, 1977, Hussain and Higuchi, 1980), *E.malabaricus* (Ruangpunit *et al.*, 1986), *E.salmoides* (Kungvankij *et al.*, 1986), *E.fuscoguttatus* (Lim *et al.*, 1990), *E.suillus* (Toledo *et al.*,1993), *E.polyphekadion* and *Cromileptis altivelis* (Sugama and Ikenoue 1999) .

5.2.1. Materials and methods.

The grouper broodstock for the present study was developed by growing fingerlings caught from wild. The grouper fingerlings taken from off Tuticorin were

transported in oxygenated bags and stocked in indoor 5 ton capacity FRP tanks at the onshore rearing facility of the Central Marine Fisheries Research Institute at Cochin Fisheries Harbour **(Plate XX).** These tanks are cylindro-conical in shape, with smooth interior and sea-blue in colour, provided with recirculating seawater using *in situ* biofilters (2 to 3 numbers). Initial stocking was done at a rate of 4 nos /m^3. The fingerlings were given prophylactic treatment before stocking. Later, they were treated whenever there was an occurrence of bacterial, fungal or parasitic infection. Bacterial diseases mainly vibriosis was frequently encountered, especially during summer months. This was controlled by giving bath treatment with oxytetracycline at a rate of 1gm/50 liter seawater for 1 hour duration, twice a day, continuing for 4 days. The fingerlings were fed with small sciaenids, nemipterids, goatfishes and small cephalopods, taken from trawl catches, twice a day at an average of 10% of their body weight, in the initial stages; after one year the fishes were fed at a rate of 4-5 % of their body weight.

Seawater was pumped from the adjoining Mattancherry canal at the peak of high tide. The salinity in the tanks was maintained between 28 and 32 ppt, temperature between 26.5° and 29°c, pH was between 7 and 8 and an optimum dissolved oxygen in the range of 4 -4.5 ml/L. Tanks were covered to reduce disturbances and artificial hideouts were provided inside the tanks . The biofilters served filtration and removal of nitrogenous wastes from the metabolites and in recirculating the water within. From January 1998 onwards, the fishes were fed at a rate of 2% of the body weight; the regular feed was enriched with cod-liver oil and vitamin E. The fishes were periodically examined for gonadial conditions through biopsy. Care was taken to ensure that the

PLATE XX

a. Grouper broodstock rearing system

fishes remained free of pathogens. They were treated (dip or bath) with 10-20 ppm furacin (9.3% nitrofurazone), for controlling bacterial infection and 100 ppm formalin for other ectoparasitic infections.

Broodstock were maintained in the same indoor tanks in sea water of salinity 32ppt, dissolved oxygen >4.0ppm and ammonia N-level 0.01- 0.02ppm. Temperature in the system ranged from 26-29°C and pH was maintained between 7.8 and 8.3. The height of water column in the tanks was restricted at 1.1m. Light intensity at the broodstock tanks varied between 350-400 lux.**(Fig.10)**

Broodstock were fed on trash fish including small squids, cuttle fish and octopus, supplemented with Vit.E, Vit.B12, ascorbic acid and sea cod for providing essential enrichment of 20:5ω3 eicosapentaenoic acid (EPA), 22:6ω3 docosahexaenoic acid (DHA) and polyunsaturated fatty acids. Feeding was *ad libitum* once around 10 a m daily. The remains of feed and faecal matter were siphoned out and the water lost was replaced with fresh seawater. Gonadal maturity of the females was monitored by inserting a cannula of 1.5mm I.D, through the urinogenital opening and biopsy examination of the gonads were carried out. A mature female was identified when vitellogenic eggs were obtained on biopsy.

Male brooder development

Male spawners were developed through hormonal sex inversion of females having total length ranging from 53 - 72cm . The male hormone 17 α methyl testosterone,

purchased from Ms. Argent Chemicals, USA, was made into pellets using cholestrol matrix and a cellulose binder (Sherwood *et al.,* 1988). The hormone pellets implanted into trash fish were administered orally to the selected females three times a week at the normal feeding time. Hormone was administered orally at an average dose of 3mg/kg body weight and the fishes were examined periodically for the presence of milt. A gentle pressure on the abdomen of mature males showed presence of milt. Diet treatment was completely successful when fish were individually fed. Stocking density was strictly restricted to 1male : 1 female or 1male : 2 females. Photoperiod regime followed was 8 L:16 D for gonad development .

5.2.2. Results

Female spawners measured 585.4±2.8mm to 720.2±1.8mm in total length and 3798.6± 2.4g to 6202.4± 3.4g in body weight; males were of 538.7± 4.8mm and 721.2± 3.9mm total length and body weight 3247.7±4.9g and 7098.3±2.8g respectively. Successful egg fertilization was obtained by using milt from sex-inverted males, with very high fertility rate of upto 99% in the present study.

Natural spawning experiments of the greasy grouper *E. tauvina* were carried out during October 1998 to July 1999 and again from October 1999 to December 2000, at the Fisheries Harbour Laboratory of the Central Marine Fisheries Research Institute at Cochin.

5.3. Fecundity.

The fecundity of a fish is often defined as the total number of ripe eggs produced by one female in a spawning season or in a year; or the capacity of the fish in terms of egg production (Bagenal, 1978). Assessment of fecundity is of paramount importance in fisheries management as it provides knowledge about the reproductive capacity of the species and the number of offsprings produced in a season (Rajasree and Kurup, 2004). The fecundity of a fish is not a stable character, but varies according to the species and to the changes in the environmental conditions. There may also be changes in fecundity of one and the same species in different years and also in different localities (Nikolsky, 1963) . Fecundity (total potential fecundity) "F"is defined according to Bagenal (1978) as "number of ripe ova present in the ovary immediately before spawning" and therefore easy to determine the fecundity if the fish spawns only once a year. But for fishes which release their eggs in several successive batches during the course of the reproductive cycle, Aboussouan and Lahaye (1979) have defined the total fecundity as " the number of oocytes destined for spawning". Fecundity aspect of reproduction is deeply associated with the studies of population dynamics and fishery management practices.

5.3.1. Materials and Methods

In the present study, ripe ovaries taken from females in the spawning season were used. Fecundity, the number of eggs released by an individual fish during a spawning was determined from 23 fishes of *E.tauvina* in stages IV and V. Length and weight of fish collected were measured in fresh condition. Ovaries were dissected out and preserved in 8% formalin. The ovaries were taken out and kept on a filter paper for

30 minutes to drain out and evaporate the excess water absorbed by them. The weight and volume of the preserved ovaries were recorded. Subsamples taken from anterior, middle and posterior regions of both ovaries were mixed randomly and subjected to volumetric and gravimetric counts. The subsamples were placed in Gilson's fluid for nearly six months with periodic shaking to release the eggs . The yolked and transparent eggs were counted . Total number of eggs or the absolute fecundity (F) was calculated using the formula F= W/ w.X , where F = absolute fecundity, W is the weight of ovaries, w is weight of sub-samples and X is the estimated number of yolked and transparent eggs in the sub-samples . This observed absolute fecundity was then related to the total length and total weight of the fish.

5.3.2. Results

The absolute fecundity denoting the total number of eggs in the ovary and the relative fecundity denoting the number of eggs per unit length or weight of the fish were also estimated.

For fecundity studies, only the yolked or the large transparent oocytes from stage IV which have begun vitellogenesis and are thus liable to be spawned are studied. The yolked eggs were rounded and opaque, yellow in colour with diameters ranging between 0.35mm to 0.6mm. The larger transparent eggs were more advanced having diameters ranging from 0.7 to 0.9mm. The number of ripe ova or the absolute fecundity of *E tauvina* in the present wstudy was found to vary from 21,17264 to 38,98465 /fish /spawn, in fish with total length ranging from 490mm to 700mm and with a body weight between 1500gm to 6712 gm. (**Table. 11**)

Fecundity in relation to length:

The relationship between absolute fecundity and length is generally said to be curvilinear (Bagenal, 1971), being represented by the following formula:

$$F=aL^b$$

Where Fis the absolute fecundity, L is the length of fish in cm and a and b are constants.

The logarithmic transformation of the above equation is a straight line relationship in the form , F= log a +b log L.

In the present study, it is seen that the Log total length (cm.) – Log absolute fecundity relationship gave a high correlation coefficient and the formula representing this relationship is as follows:

F = 0.336 +L^2.49

This relatioship is represented graphically in figure **9.**

(**Table 11**) shows the mean observed and relative fecundity per total length group of *E.tauvina.* The relative fecundity ranges from 15607.27 to 57755.03 per cm.

Fecundity in relation to body weight:

The equation F =1.45938 +W^0.79 represents the total weight (gm) –absolute fecundity relationship ; where F is the absolute fecundity and Wis the total weight in gram.

The graphical representation of this linear relationship is shown **in fig 8. Table (** 11) gives the mean observed and relative fecundity per total weight group of *E. tauvina.*

Fig.8. Fecundity in relation to total weight

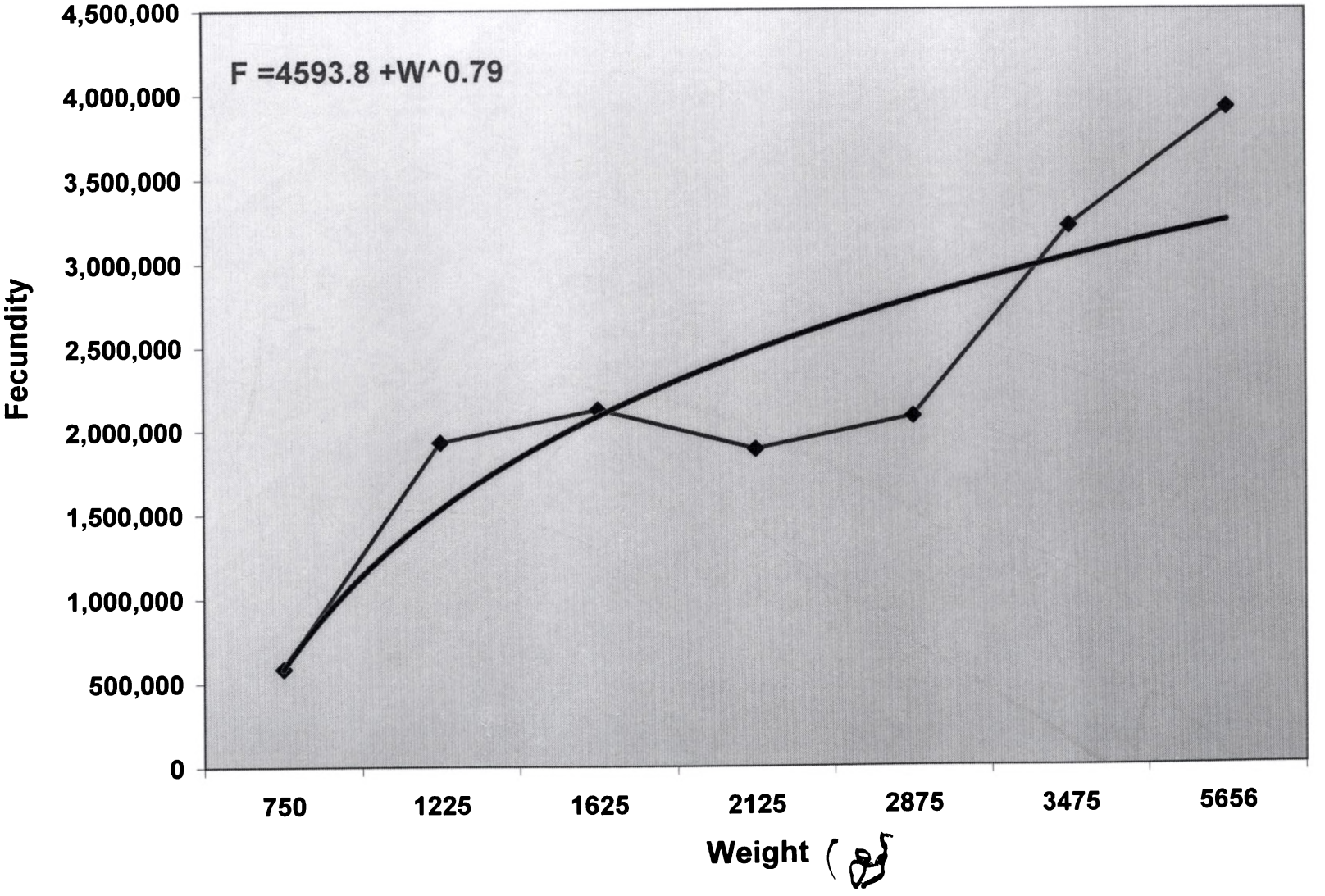


Fig 9. Fecundity in relation to Total Length

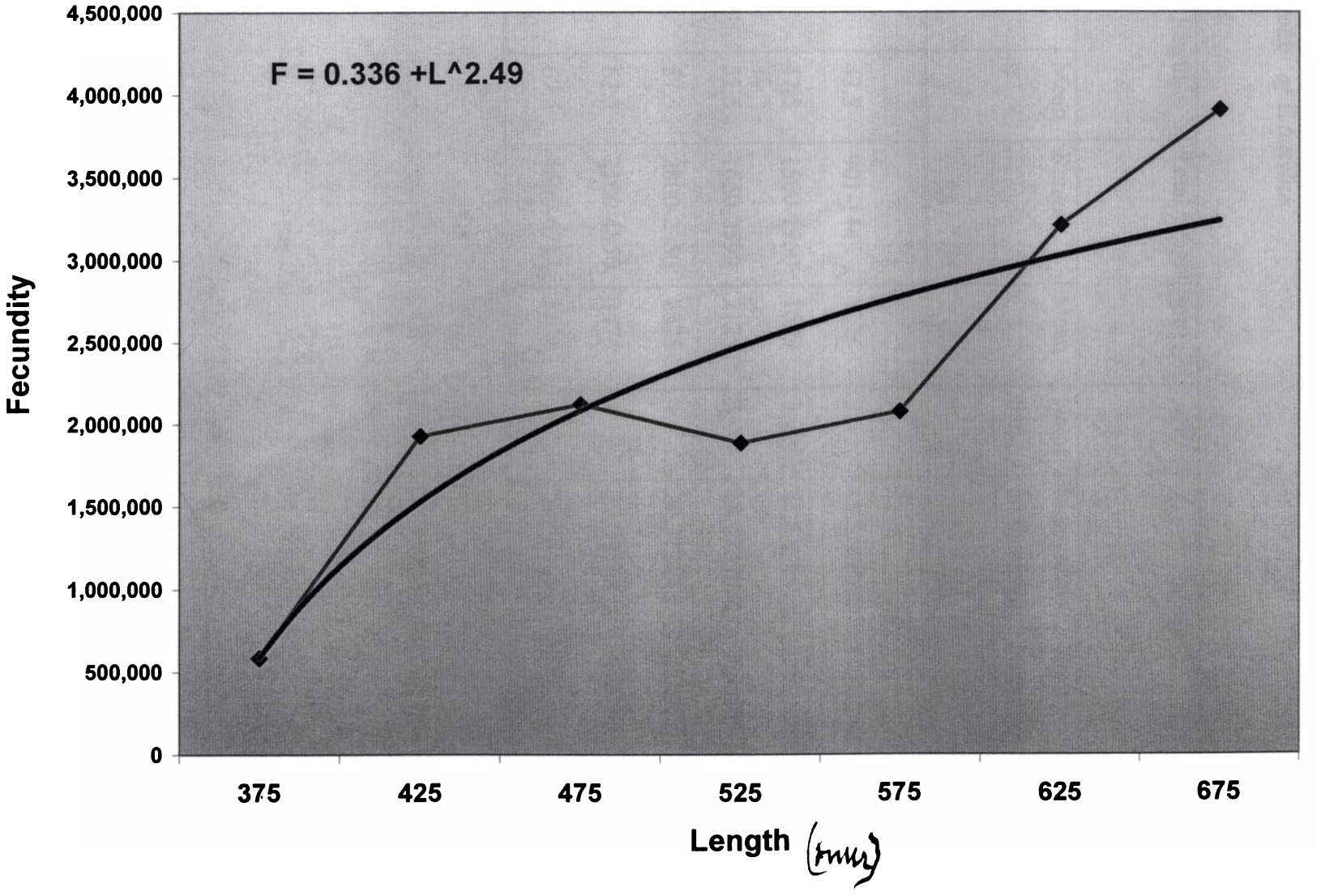


Table. 11 ***Mean observed and relative fecundity per total length and total weight group of E.tauvina***

Standard length(mm)		Total weight (gm)					
Range	Mean	Range	Mean	No.of fish	Meanobserved fecundity	Relative fecundity (F/gm.)	Relative fecundity (F/cm)
40.-44.9	42.5	901-1449	1225	3	19,25920.4	1572.180	45315.77
45-49.9	47.5	1500-1750	1625	3	21,17264	1302.931	44573.98
50-54.9	52.5	1650-2599	2125	3	18,77370.8	883.469	35759.44
55-59.9	57.5	2800-2950	2875	4	20,71214.	720.422	36021.11
60-64.9	62.5	2100-4850	3475	2	32,04532.	922.167	51272.51
65-69.9	67.5	4600-6712	5656	2	38,98465	689.261	57755.03

From this table it is noted that the values of the relative fecundity ranges from 689.261 to 1572.180 per gram.

The absolute fecundity of *E tauvina* in the present wstudy was found to vary from 21,17264 to 38,98465 /fish /spawn; which increases with increase in total length and total weight . Similar relationships between fecundity and length/weight range have also been reported for *E. tauvina* in Arabian Gulf by El-Sayed & Abdel-Bary (1999) and also for *E. chlorostigma* (Ghorab *et al.*, 1986), who also found that the fecundity of *E. chlorostigma* from the Red Sea increased with increase in total length and total weight of the fish.

5.4. Spawning.

Spawning is one of the most significant phases of the reproductive cycle, comprising of ovulation and oviposition in female and release of sperm in the female. The success or failure of spawning has direct impact on the population size, continuation and survival of the species (Agarwal 1996). According to Nikolskii (1963), the spawning timings in fishes are so precise that chances of survival of their eggs and larvae are maximal. A number of factors like endocrinological and environmental and ecological factors co-act in this. Of the many possible environmental factors, lunar periodicity, temperature, photoperiod and rainfall are important in regulating reproductive cycle in many teleosts. Besides, many social factors like sex ratio, courtship behaviour, pheromones, crowding etc. are also important in synchronizing spawning.

There are several reports on the natural as well as induced spawning of many species of groupers. Artificial spawning and larval rearing of *Epinephelus tauvina* was carried out by Chen *et al.*,(1977) in Singapore, while natural spawning of the same species was observed by Hussain and Higuchi (1980) in Saudi Arabia. Ukawa *et al.*, (1966) observed natural spawning of *E.akaara* was in tanks and Toledo *et al.*, (1993) have reported the natural spawning of *E.suillus* in captivity in a concrete tank and also in a floating net cage. In Singapore, although initial success obtained was in induced spawning, considerable progress has been made in achieving spontaneous spawning. of *E.malabaricus* and *E. akara* (Chen *et al.*, 1977). Natural spawning for *E. fuscoguttatus* (Lim *et al.*, 1990) and *E. polyphekadion* (James *et al.*, 1997), was obtained in tanks . The present study elucidates the natural spawning, egg production, egg quality etc. of *E. tauvina* brood stock reared from wild caught fingerlings in captivity in sea water recirculating culture system in indoor 5 ton capacity tanks.

5.4.1. Materials and methods.

On 29-10-1998, a mature female broodstock weighing 3.85 kg and the above artificially sex transformed mature male broodstock weighing 3.2 kg released together, spawned spontaneously for the first time, between 1700 and 2200hrs . Spawning by the same pair occurred again on 30-10-1998, between 1700 and 2000 hrs.

Natural spawning experiments of the greasy grouper *E. tauvina* were carried out six times during October 1998 to July 1999 and again six times from October 1999 to December 2000, at the laboratory at Central Marine Fisheries Research Institute at

Cochin. The spawners were maintained in cylindro-conical 5 ton FRP tanks of light blue interior colour, in re-circulating sea water of salinity 32 ppt, dissolved oxygen >4.0ppm and ammonia N-level 0.01- 0.02ppm. Temperature in the system ranged from 26-29°C and pH was maintained between 7.8 and 8.3 **(Fig.10.)** The height of water column in the tanks was restricted at 1.1m. Light intensity at the spawning tanks varied between 350-400 lux.

Female spawners measured 585.4mm to 720.2mm in total length and 3798.6g to 6202.4g in body weight; males were of 538.7mm and 721.2mm total length and body weight 3247.7g and 7098.3g respectively. Egg samples were taken from the anterior and middle portion of the ovary through gentle aspiration and the oocytes were examined and measured under a calibrated microscope. A mature female was identified when vitellogenic eggs of diameter above 450 μm were obtained on biopsy.

Male spawners were developed through hormonal sex inversion of females using the male hormone 17 α methyl testosterone. The fishes were examined periodically for the presence of milt. A gentle pressure on the abdomen of mature males showed presence of milt. Stocking density was strictly restricted to 1male : 1 female or 1male : 2 females **(Plate XVIIIa)**. Photoperiod regime followed was 8 L:16 D for gonad development .

Transparent, buoyant eggs were observed in the tanks after the spawning activity took place. Total number of eggs were estimated by counting three replicate samples

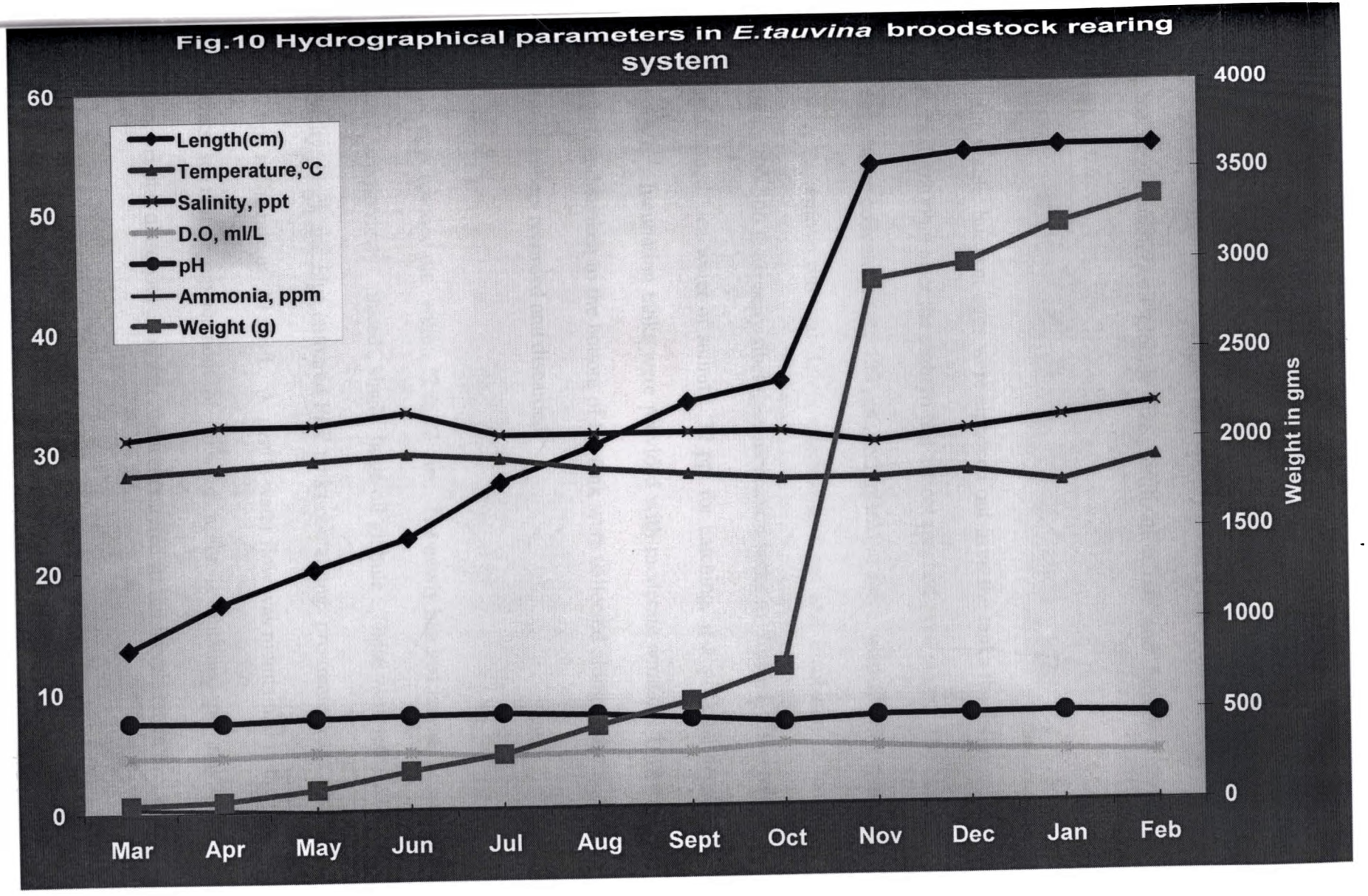


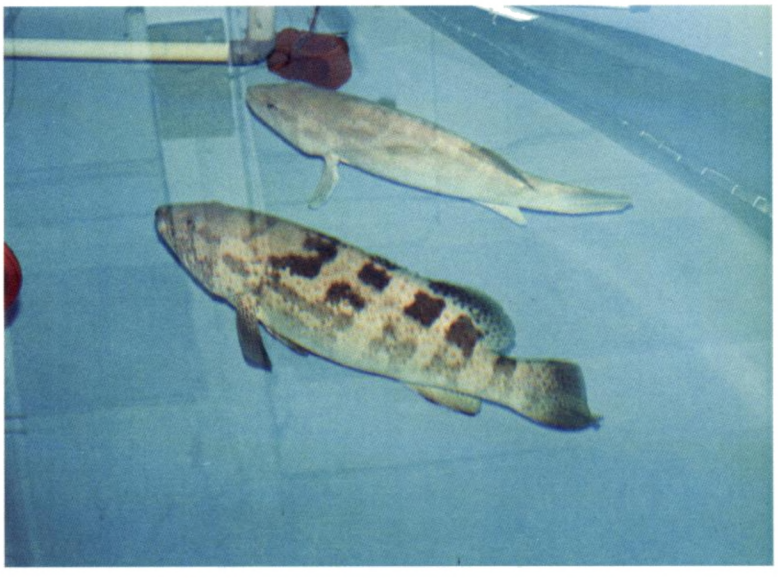
Fig.10 Hydrographical parameters in E.tauvina broodstock rearing system
Length(cm)
Temperature,°C
Salinity, ppt
D.O, ml/L
pH
Ammonia, ppm
Weight (g)
60
50
40
30
20
10
0
4000
3500
3000
2500
2000
1500
1000
500
0
Weight in gms
Mar
Apr
May
Jun
Jul
Aug
Sept
Oct
Nov
Dec
Jan
Feb

from the tank water. The number of viable, fertilized floating eggs and the unfertilized sunken eggs were estimated by taking aliquots in a measuring jar and counting them. Samples of fertilized eggs were examined periodically under a compound microscope for recording the egg diameter, oil globule size, fertilization rate and further embryonic development.

All the viable, buoyant eggs were siphoned out from the tanks the following day morning at about 0800hrs after the embryo has almost reached eyed stage. The buoyant fertilized eggs were collected using 400 μm mesh plankton sieve, washed with clean sea water in order to remove dirt and debris attached to them and incubated at ambient temperature in 300-500 L capacity fiberglass incubation tanks in filtered, UV - treated , and pre-conditioned sea water of salinity 32 ppt for hatching, at a stocking density of 250-300 eggs L^{-1}. Incubation tanks were provided with moderate aeration. Unfertilized opaque eggs found sunken to the bottom of the tank were collected along with the debris and effluent, numbers recorded and discarded.

The eggs hatched out within 22 to 23 hrs. The newly hatched larvae possessed yolk sac and a posteriorly placed , single large oil globule . These were released into 300L capacity black and blue coloured FRP tanks containing pre-treated sea water of salinity 32 ppt, at the rate of 30 nos/L. A gentle water flow was maintained in the larval rearing tanks so that a replacement of 10% of water is obtained per day. Water temperature, pH and dissolved oxygen were monitered in the larval tanks every three hours.

PLATE XVIII



a. Grouper broodstock

b. Pre-spawning play inside the spawning tank.

5.4.2 Results

From the12 instances of natural spawning during the present experimental period, 5.2 million eggs were collected in all, with an average fertilization rate of 90%. Natural spawning by spawners of *E. tauvina* occurred 6 times during October 1998 to July 1999 period and 6 times during October 1999 to December 2000. On every occasion, the spawning run by a single pair lasted for 2 or 3 days. At the time of spawning the male developed a whitish pale colour while the female was greenish brown above and white below. Actual spawning behaviour started from 5.00 p.m and continued after sunset up to 8.00 p.m. Prior to spawning, the spawning pair swam together, contacting each other . The pair ascended rapidly in the water column with anterior part of their bodies exposed and released the gametes while dashing into the water.

Captive spawning of *E. tauvina* in the indoor 5 ton FRP tank in re-circulating sea water occurred for the first time on 29th October 1998, four days before full moon and continued up to 30th . The female measured 591.7± 2.5mm in total length and weighed 3848.6±3.3g. The male fish was of total length 543.5 ±3.7mm and total weight 3247.7±3.2g. Subsequent spawning by the same pair in December coincided with the new moon day. During May 1999, the same pair of spawners spawned on 16th which was a new moon day; after a gap of four days again the they started spawning on 23rd, in the last quarter moon phase and continued up to 25th . Spontaneous spawning which occurred in June as well as December coincided with the full moon phase and the last quarter before full moon respectively. In the present study; on most occasions there was

close correlation of spontaneous spawning with the lunar phase; spawning occurred either during the last phase of the lunar cycle (41.6%) or on days of full moon (25%) or new moon (33.3%). However, there was no significant correlation between the number of eggs released and lunar periodicity on any of the occasions during the present study. A single female of size 619.6±3.8mm total length and 4878.2±2.6g weight, consistently released eggs during May, June and July 1999. Egg production ranged from 0.29 million to 0.5 million during the present set of spawning experiments. The females released an average of 50,428 numbers of eggs /kg body weight on each day of spawning

Pre-spawning play or courtship started five to six days before the actual spawning started; the spawning pair swam together, contacting each other **(Plate XVIII** b). The female spawner could be distinguished by the presence of heavily bulged belly and a pinkish coloured vent and the male spawner developed more yellowish coloration. At the time of spawning the male developed a whitish pale colour while the female was greenish brown colour. The fishes spawned naturally, actual spawning behaviour started from 3.00 p.m onwards and continued after sunset up to 8.00 p.m. On every occasion, the spawning run by a single pair lasted for 2 to 4 days. The pair ascended together rapidly in the water column with head and anterior part of their bodies protruding and exposed above the water column and released the gametes while dashing into the water. A single female of size 619.6mm total length and 4878.2g weight, consistently released eggs during May, June and July 1999. From the12 instances of natural spawning during the present experimental period, 5.2 million fertilized eggs were collected in all, with an

average fertilization rate of 90%. Natural spawning by spawners of *E. tauvina* occurred 6 times during October 1998 to July 1999 period and again 6 times during October 1999 to December 2000.

Effect of Lunar Cycle on Spawning

There are several reports on the role of lunar periodicity in the synchronous spawning of groupers (Quinitio *et al.*, 1977, Johannes 1978, Smith 1972, Toledo *et al.*, 1993, Lee *et al.*, 2002, Sudaryanto *et al.*, 2004); *Epinephelus striatus* spawns around new moon in the Bahamas while *E.merra* and *E. striatus* spawn around the full moon phase in the Society Islands and off Belize. *E. coioides* held in tanks and cages spawn usually within four days before or after the last quarter moon phase. Three species of groupers(*E.fuscoguttatus, E.coioides* and *Cromileptes altivelis*) stocked at Komodo in floating cages were found to spawn around the period of new moon (Sudaryanto *et al.*, 2004).

Lunar cycle was seen to affect spawning activity of groupers *Epinephelus tauvina* in the present study. It has also been observed that *Epinephelus tauvina* spawned in FRP tanks placed indoors almost every month usually within 4 days before or after the last quarter moon phase; at this stage ovaries contained oocytes at the peri-nucleolus and oil droplet stages; fresh ovulatory follicles were also observed in some around this period. Spawning peak occurred during second lunar quarter (days 10-12), and spawning was completed at about full moon. Ovaries observed afterwards were shrunken, flaccid, reddish in colour and contained empty follicles and very few residual

Table. 12. *Natural spawning of E.tauvina in relation to Lunar phase**

Sl.No.	Date of spawning	Lunar day	Time
1	29-10-1998	11	1800
	30-10-1998	12	1720
2	20-12-1998	1	1700
	21-12-1998	2	1740
	22-12-1998	3	1800
3	16-5-1999	1	1700
	17-5-1999	2	1740
	18-5-1999	3	1820
			1740
4	23-5-1999	8	1730
	24-5-1999	9	
	25-5-1999	10	1750
			1820
5	02-6-'99	18	
	07-6-'99	23	1800
			1750
6	10-7-1999	24	1800
	11-7-1999	25	1830
7	12-7-1999	26	
			1720
	22-10-'99	26	1810
8	23-10-'99	27	1820
	24-10-'99	28	
9			1830
	22-12-'99	15	1820
10.	15-7-2000	14	1850
	16-7-2000	15	1830
11.	02-10-2000	6	1720
	03-10-2000	7	1800
12.	18-10-2000	19	1820
	19-10-2000	20	1800
	25-12-2000	1	1840

*Lunar day1represents new moon, 14 represents full moon

ova. In the present study, on most occasions there was close correlation of spontaneous spawning with the lunar phase; spawning occurred either during the last phase of the lunar cycle (41.6%), 33.3% of the spawning took place around new moon or (25%) on days of full moon **(Table.12)**.

As reviewed by Claydon (2004) in other reef fishes, lunar synchronization presumably serves to coordinate gamete maturation. It was observed in the present study that in *E.tauvina* , in females, peaks in Gonado - Somatic Index and mean ova diameter synchronized with lunar cycle; further, hydrated oocytes were found during successive new-moon phases. These results suggest that gonadal development in females *E.tauvina* has been influenced by lunar periodicity which was observed in the actual captive spawning obtained in indoor tanks during the present study.

Effect of lunar rhythm on reproduction has been reported for *E. tauvina* in Arabian Gulf by El-Sayed and Abdel-Bary (1999) while studying their reproductive biology. Similar effects of lunar rhythm on grouper reproduction have been reportedon *E.guttatus* in Peurto Rico (Rosario, 1989) and that these fishes aggregate for spawning the week before full moon (end of first quarter), while spawning takes place 1-2 days after full moon.

Quality of eggs and egg production

Transparent, pelagic, spherical, buoyant and non-adhesive eggs were observed in the tanks after the spawning activity took place. Aliquot samples were examined

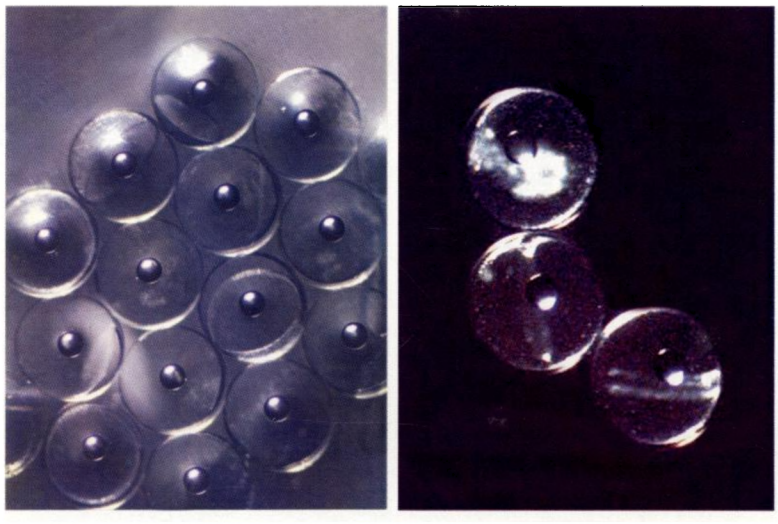
periodically under a compound microscope for recording the egg diameter, oil globule size, fertilization rate and further embryonic development. Eggs were transparent, unpigmented and spherical with a smooth chorion , vitelline membrane and unsegmented yolk, ova diameter ranged from 780μm to 910 μm, with a single oil globule, measuring upto 190μm **(Plate XIXa).** Yolk was colourless, transluscent, unpigmented and homogenous. Good quality eggs possessed a single large oil globule. Fertilization rate obtained in the present study was 85-99%. Total number of eggs were estimated by counting three replicate samples from the tank water. All the viable, buoyant eggs were siphoned out from the tanks the following day morning at about 0800hrs after the embryo has almost reached eyed stage. Unfertilized opaque eggs found sunken to the bottom of the tank were collected along with the debris and effluent, numbers recorded and discarded.

Egg production ranged from 0.29 million to 0.5 million during the present set of spawning experiments. The females released an average of 50,428 numbers of eggs /kg body weight on each day of spawning **(Fig.10).** During all the spawning runs in these experiments the eggs released were of good quality in terms of buoyancy and fertilization rate **(Fig.11)**

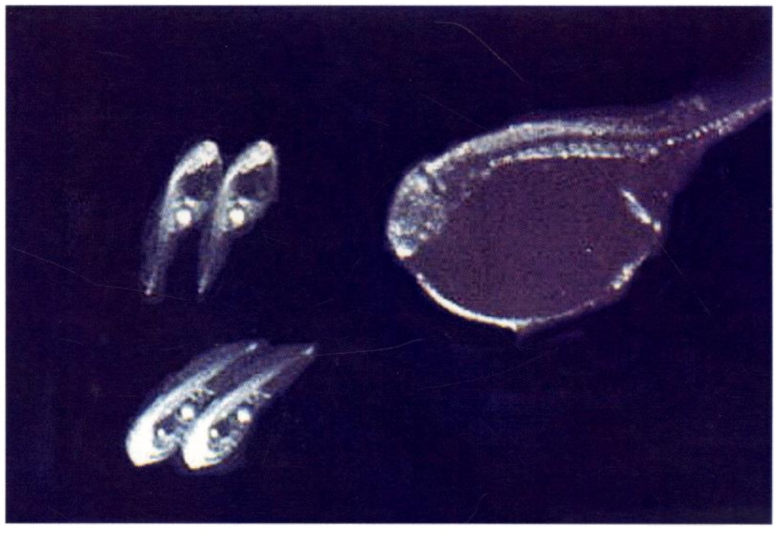
5.5. Incubation and hatching.

The buoyant eggs were collected using 400 μm mesh plankton sieve. Eggs were washed with clean sea water in order to remove dirt and debris attached to them. These eggs were then incubated at ambient temperature in 300-500 L capacity fiberglass

PLATE XIX



a. Fertilized eggs in stages of development



b. Newly hatched out grouper larvae

5.4.2 Results

From the12 instances of natural spawning during the present experimental period, 5.2 million eggs were collected in all, with an average fertilization rate of 90%. Natural spawning by spawners of *E. tauvina* occurred 6 times during October 1998 to July 1999 period and 6 times during October 1999 to December 2000. On every occasion, the spawning run by a single pair lasted for 2 or 3 days. At the time of spawning the male developed a whitish pale colour while the female was greenish brown above and white below. Actual spawning behaviour started from 5.00 p.m and continued after sunset up to 8.00 p.m. Prior to spawning, the spawning pair swam together, contacting each other . The pair ascended rapidly in the water column with anterior part of their bodies exposed and released the gametes while dashing into the water.

Captive spawning of *E. tauvina* in the indoor 5 ton FRP tank in re-circulating sea water occurred for the first time on 29th October 1998, four days before full moon and continued up to 30th . The female measured 591.7± 2.5mm in total length and weighed 3848.6±3.3g. The male fish was of total length 543.5 ±3.7mm and total weight 3247.7±3.2g. Subsequent spawning by the same pair in December coincided with the new moon day. During May 1999, the same pair of spawners spawned on 16th which was a new moon day; after a gap of four days again the they started spawning on 23rd, in the last quarter moon phase and continued up to 25th . Spontaneous spawning which occurred in June as well as December coincided with the full moon phase and the last quarter before full moon respectively. In the present study; on most occasions there was

close correlation of spontaneous spawning with the lunar phase; spawning occurred either during the last phase of the lunar cycle (41.6%) or on days of full moon (25%) or new moon (33.3%). However, there was no significant correlation between the number of eggs released and lunar periodicity on any of the occasions during the present study. A single female of size 619.6±3.8mm total length and 4878.2±2.6g weight, consistently released eggs during May, June and July 1999. Egg production ranged from 0.29 million to 0.5 million during the present set of spawning experiments. The females released an average of 50,428 numbers of eggs /kg body weight on each day of spawning

Pre-spawning play or courtship started five to six days before the actual spawning started; the spawning pair swam together, contacting each other **(Plate XVIII b)**. The female spawner could be distinguished by the presence of heavily bulged belly and a pinkish coloured vent and the male spawner developed more yellowish coloration. At the time of spawning the male developed a whitish pale colour while the female was greenish brown colour. The fishes spawned naturally, actual spawning behaviour started from 3.00 p.m onwards and continued after sunset up to 8.00 p.m. On every occasion, the spawning run by a single pair lasted for 2 to 4 days. The pair ascended together rapidly in the water column with head and anterior part of their bodies protruding and exposed above the water column and released the gametes while dashing into the water. A single female of size 619.6mm total length and 4878.2g weight, consistently released eggs during May, June and July 1999. From the12 instances of natural spawning during the present experimental period, 5.2 million fertilized eggs were collected in all, with an

average fertilization rate of 90%. Natural spawning by spawners of *E. tauvina* occurred 6 times during October 1998 to July 1999 period and again 6 times during October 1999 to December 2000.

Effect of Lunar Cycle on Spawning

There are several reports on the role of lunar periodicity in the synchronous spawning of groupers (Quinitio *et al.*, 1977, Johannes 1978, Smith 1972, Toledo *et al.*, 1993, Lee *et al.*, 2002, Sudaryanto *et al.*, 2004); *Epinephelus striatus* spawns around new moon in the Bahamas while *E.merra* and *E. striatus* spawn around the full moon phase in the Society Islands and off Belize. *E. coioides* held in tanks and cages spawn usually within four days before or after the last quarter moon phase. Three species of groupers(*E.fuscoguttatus, E.coioides* and *Cromileptes altivelis*) stocked at Komodo in floating cages were found to spawn around the period of new moon (Sudaryanto *et al.*, 2004).

Lunar cycle was seen to affect spawning activity of groupers *Epinephelus tauvina* in the present study. It has also been observed that *Epinephelus tauvina* spawned in FRP tanks placed indoors almost every month usually within 4 days before or after the last quarter moon phase; at this stage ovaries contained oocytes at the peri-nucleolus and oil droplet stages; fresh ovulatory follicles were also observed in some around this period. Spawning peak occurred during second lunar quarter (days 10-12), and spawning was completed at about full moon. Ovaries observed afterwards were shrunken, flaccid, reddish in colour and contained empty follicles and very few residual

Table. 12. *Natural spawning of E.tauvina in relation to Lunar phase**

Sl.No.	Date of spawning	Lunar day	Time
1	29-10-1998	11	1800
	30-10-1998	12	1720
2	20-12-1998	1	1700
	21-12-1998	2	1740
	22-12-1998	3	1800
3	16-5-1999	1	1700
	17-5-1999	2	1740
	18-5-1999	3	1820
		8	1740
4	23-5-1999	9	1730
	24-5-1999	10	
	25-5-1999		1750
		18	1820
5	02-6-'99	23	
	07-6-'99		1800
		24	1750
6	10-7-1999	25	1800
	11-7-1999	26	1830
7	12-7-1999		
		26	1720
	22-10-'99	27	1810
8	23-10-'99	28	1820
	24-10-'99		
9		15	1830
	22-12-'99		1820
		14	
10.	15-7-2000	15	1850
	16-7-2000		1830
		6	
11.	02-10-2000	7	1720
	03-10-2000		1800
		19	
12.	18-10-2000	20	1820
	19-10-2000		
	25-12-2000	1	

*Lunar day1represents new moon, 14 represents full moon

ova. In the present study, on most occasions there was close correlation of spontaneous spawning with the lunar phase; spawning occurred either during the last phase of the lunar cycle (41.6%), 33.3% of the spawning took place around new moon or (25%) on days of full moon **(Table.12)**.

As reviewed by Claydon (2004) in other reef fishes, lunar synchronization presumably serves to coordinate gamete maturation. It was observed in the present study that in *E.tauvina* , in females, peaks in Gonado - Somatic Index and mean ova diameter synchronized with lunar cycle; further, hydrated oocytes were found during successive new-moon phases. These results suggest that gonadal development in females *E.tauvina* has been influenced by lunar periodicity which was observed in the actual captive spawning obtained in indoor tanks during the present study.

Effect of lunar rhythm on reproduction has been reported for *E. tauvina* in Arabian Gulf by El-Sayed and Abdel-Bary (1999) while studying their reproductive biology. Similar effects of lunar rhythm on grouper reproduction have been reportedon *E.guttatus* in Peurto Rico (Rosario, 1989) and that these fishes aggregate for spawning the week before full moon (end of first quarter), while spawning takes place 1-2 days after full moon.

Quality of eggs and egg production

Transparent, pelagic, spherical, buoyant and non-adhesive eggs were observed in the tanks after the spawning activity took place. Aliquot samples were examined

periodically under a compound microscope for recording the egg diameter, oil globule size, fertilization rate and further embryonic development. Eggs were transparent, unpigmented and spherical with a smooth chorion , vitelline membrane and unsegmented yolk, ova diameter ranged from 780μm to 910 μm, with a single oil globule, measuring upto 190μm **(Plate XIXa).** Yolk was colourless, transluscent, unpigmented and homogenous. Good quality eggs possessed a single large oil globule. Fertilization rate obtained in the present study was 85-99%. Total number of eggs were estimated by counting three replicate samples from the tank water. All the viable, buoyant eggs were siphoned out from the tanks the following day morning at about 0800hrs after the embryo has almost reached eyed stage. Unfertilized opaque eggs found sunken to the bottom of the tank were collected along with the debris and effluent, numbers recorded and discarded.

Egg production ranged from 0.29 million to 0.5 million during the present set of spawning experiments. The females released an average of 50,428 numbers of eggs /kg body weight on each day of spawning **(Fig.10).** During all the spawning runs in these experiments the eggs released were of good quality in terms of buoyancy and fertilization rate **(Fig.11)**

5.5. Incubation and hatching.

The buoyant eggs were collected using 400 μm mesh plankton sieve. Eggs were washed with clean sea water in order to remove dirt and debris attached to them. These eggs were then incubated at ambient temperature in 300-500 L capacity fiberglass

Fig.11. Egg production during natural spawning

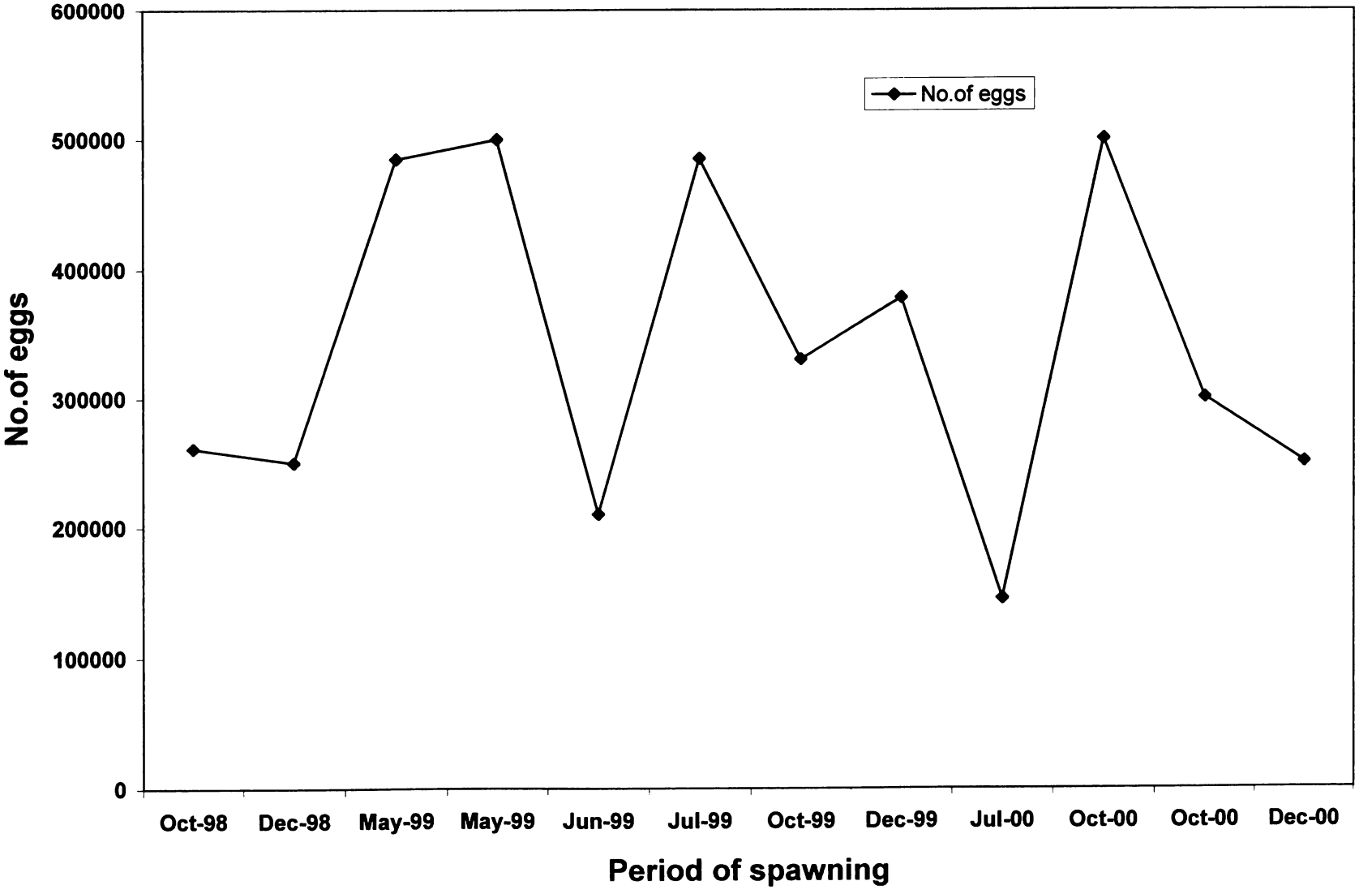
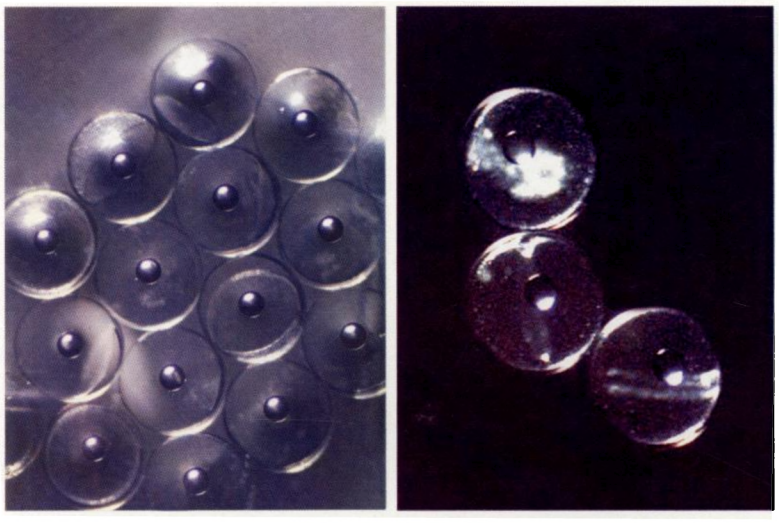


PLATE XIX



a. Fertilized eggs in stages of development

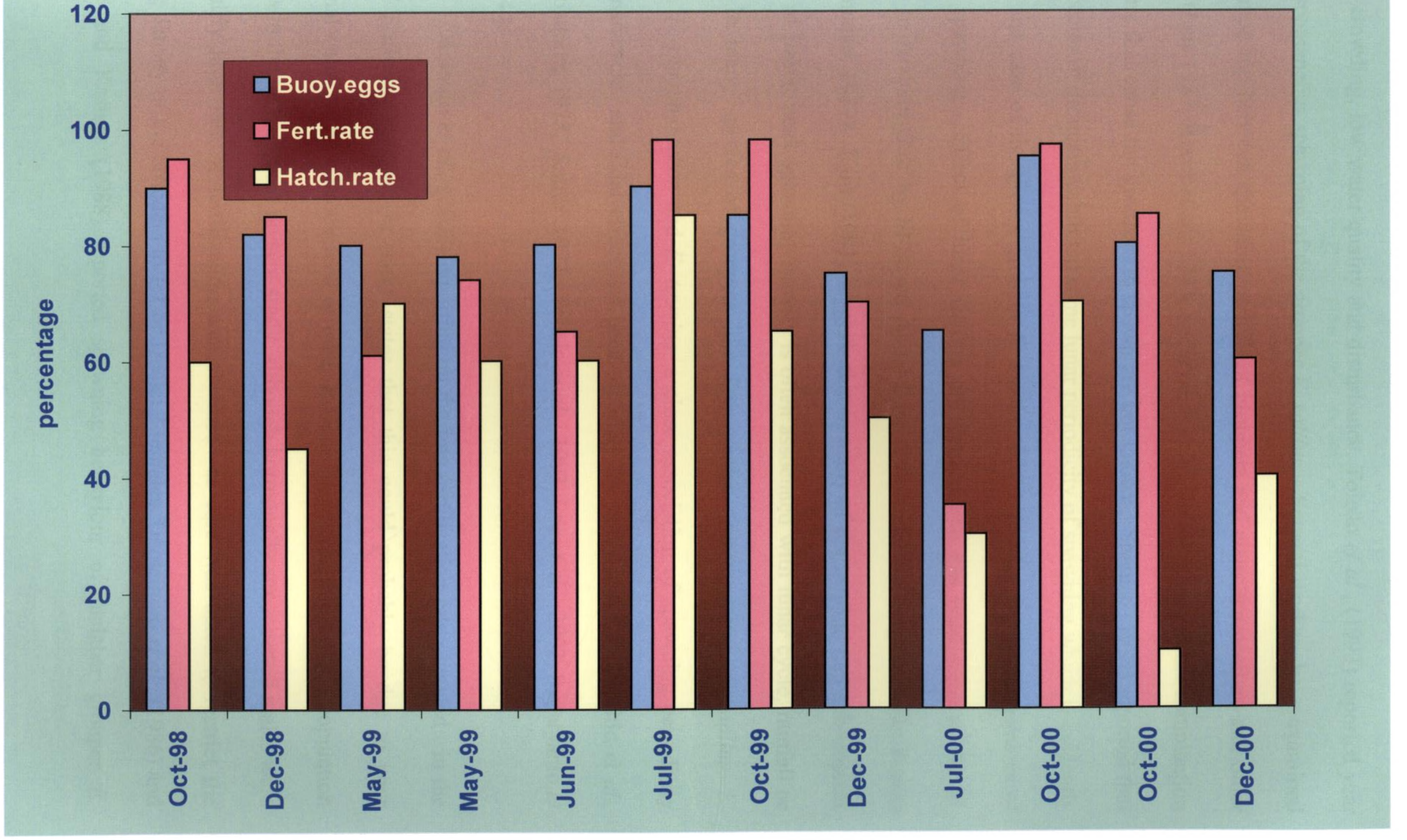
b. Newly hatched out grouper larvae

tanks, in double filtered, u.v.treated pre-conditioned sea water of salinity 32ppt, at a stocking density of 250-300 eggs L^{-1}. Incubation tanks were provided with moderate aeration. Dead eggs were siphoned out from the bottom of incubation tank every 2-3 hours. By 16 hours the embryo appeared as a cylindrical rod pressing into the surface of the yolk, the eyes and auditory sacs as well as formation of myomeres could be seen. Well developed embryos with eyes were observed at 17-18 hrs of incubation . Eggs hatched out in 22-23 hours at a temperature of 28-30° C. The newly hatched out yolk- sac larvae measured 1.6 to 1.7mm in length. **(Plate XIXb)** . The yolk sac on the anterior ventral side of the body occupied almost half of the total body length. The single oil globule measured upto 190μm. Yolk was colourless, transluscent, unpigmented and homogenous. Egg hatching rate (up to 80%), obtained in the present investigations are much higher than that obtained for the same species in Kuwait, where fertilization rate was 9% and hatching rate 24% only (Hussain and Higuchi, 1980). Temperature influences incubation time of grouper eggs. The fairly high hatching rate obtained reflects the health of the spawners. **(Fig.12)**

5.6. Discussion.

This study shows that the greasy grouper is capable of spawning naturally under captive conditions as already reported by Hussain and Higuchi (1980), although the earlier reports on natural spawning are either in ponds or in tanks of very large capacity. Tucker (1994) in his review on spawning by captive serranid fishes, states that many serranids will reproduce voluntarily if they are well nourished and protected from stress -

Fig.12. Quality of eggs produced during natural spawning



mainly crowding, low water quality and disturbance. Toledo *et al.*, (1993) reported year round spontaneous spawning of the grouper *E. suillus*. Several workers have examined the relationship between spawning periods of groupers and water temperature and other factors (Leis 1987, Alava *et al.*, 1993, Sudaryanto *et al.*, 2004) but the exact combination of factors that cause the groupers to spawn are not known. Shapiro (1987) reported that most species of groupers exhibited some lunar periodicity of spawning which is evidently seen in the case of the greasy grouper *E. tauvina* in the present set of experimcnts of natural spawning. Effect of lunar rhythm on reproduction has been reported for *E. tauvina* in Arabian Gulf by El-Sayed Abdel-Bary (1999) and for *E. guttatus* in Puerto Rico(Jimenez 1989). Lam (1983) suggests that in many of the tropical and sub-tropical species of fishes, peak spawning activity is often associated with lunar cycle, rainfall or floods. In many species of groupers like *Epinephelus fuscoguttatus, E. suillus, E. striatus, E. polyphekadion* and *E. tauvina,* a close association of spawning periodicity with lunar cycle and tidal effect has been reported by many authors (Quinitio *et al.*, 1977, Johannes 1978, Smith 1972, Toledo *et al.*, 1993, Lee *et al.*, 2002, Sudaryanto *et al.*, 2004).

Okamura *et al.*, (2002) reporting on the low fertilization rate obtained in the case of *E.akaara* cultured in tanks states that this could probably be due to poor nutritional value of broodstock feed or other reasons like stress due to high population densities in the tanks. In the present study, although hormonally sex inverted males were used, fairly high rate of fertilization was obtained as care was taken to restrict the stocking density of the brooders to 1:1 or 1male: 2 females. Hamamoto *et al.*, (1986) and Manabe and Kasuga (1988) reported spawning behaviour of malabar grouper *E.*

malabaricus and *E. moara* in aquarium tanks of 2m depth where the spawning pairs swam at the surface with their heads out of water prior to spawning. Egg fertilization rate (up to 99%), and hatching rate (up to 80%), obtained in the present investigations are much higher than that obtained for the same species in Kuwait, where fertilization rate was only 9% and hatching rate 24% (Hussain and Higuchi, 1980) ; Manabe and Kasuga (1988) reported on fertilization rate obtained under 5% which could be due to the limited space in the tank.

Chen *et al.*, (1977) reported the diameter of fertilized eggs of *E. tauvina*, as 0.90 mm and total length of the newly hatched larva as 1.70 mm., which is almost similar to that obtained in the present study. Hussain *et al.*, (1975) have reported the average diameter of egg as 0.77 mm, and that the newly hatched larvae measured 1.4 to 1.5 mm. In subsequent studies, Hussain and Higuchi (1980) recorded the total length of newly hatched larvae of *E. tauvina*, as 2.25mm. In Singapore, Lim (1993), recorded the average egg diameter as 0.80mm, for *E. tauvina.* The variations observed in the size of eggs and larvae may be attributed to the condition of the broodstock, type of spawning and also to the season of spawning. It has been found that fertilized eggs of groupers vary between 700 and 1200μ m in size but without any morphological variations. There is significant correlation between the egg diameter and subsequent development, larval size and survival potential (Bagarinao and Chua 1986). Lam (1983) and Chao and Chow (1990) have pointed out that high levels of D H A and E PA are essential in the brood stock diet to obtain good quality eggs as well as to enhance larval survival in *E. tauvina.* Tucker (1994) reviewing spawning of captive serranids, states that egg quality could vary

with the type of spawning, condition of broodstock, spawning season, size and age of the female. Good nutrition, especially proper quality and quantity of fat eaten by females are important to ensure high quality of eggs. In general, eggs obtained from natural spawning are bigger in size and better in quality than those obtained through induced ovulation. Viability of artificially spawned eggs is also likely to fluctuate.

Chapter VI.

Grouper culture.

6.1 Introduction.

Between 1980 and 1990, world aquaculture production increased at an average annual rate of 9.6%, five times the global population growth. (Csavas, 1994). Although aquaculture production has continued to increase Csavas (1994) predicted that the demand may not be met at the current growth rate of aquaculture production. Because of the stagnation in growth of capture fisheries, demand for seafood relied on growth in aquaculture production. Finfish production has been the major contributor to world aquaculture production. Majority of finfish aquaculture production is from freshwater environment. However, the proportion grown from marine water is increasing (36% in 2000, Ottolenghi *et al.*, 2004).

The groupers (Family Serranidae, genus Epinephelus) are an economically important group of marine fishes widely distributed in tropical and subtropical areas throughout the world. They are high-value food fish in the urban and export markets in many parts of the world particularly in the Asia-Pacific region, because of the high demand for groupers in local and export markets, and the high value they fetch in these markets. The demand for, and value of live groupers have grown markedly in the last two decades. Approximately two-thirds of this demand is met from capture fisheries of

market size fish. The development of large and affluent markets for live reef fish, especially in Hong Kong and southern China has increased pressure on wild stock resources. The high demand for live reef fish and the profitability of this trade has encouraged overfishing and also the use of destructive fishing practices particularly the use of cyanide to capture them. There is considerable potential for aquaculture production of groupers to replace wild-caught product, and to assist in alleviating environmental damage and overfishing.

Worldwide, most grouper aquaculture production is from Southeast Asia. Based on FAO data, Taiwan and Indonesia are the major producers of farmed grouper, followed by Thailand and Malayasia.

There are three major issues that have to be addressed if the trade of these fishes has to be sustained viable in future. Groupers, being slow growing are highly vulnerable to overfishing and as indications are there, in many areas overexploitation has already occurred (Cesar *et al.*,2000). The second is the use destructive fishing practices, especially cyanide fishing, and the threats these pose to habitats on which these species depend for food and shelter. As wild resources of market-size fish have depleted from many of the known grounds, buyers looked farther into new grounds especially in the Pacific oceans and the fishes bear the naturally-occurring ciguatoxins (Sadovy, 2001). There is, therefore, an urgent need to develop alternative sources of grouper to take the pressure off wild stocks, to reduce the use of cyanide-caught fish and to provide safe, ciguatera-free, fish.

As a partial solution to these problems, as a means of generating foreign exchange and enhancing livelihood options in coastal communities, there is keen interest in expanding and improving mariculture of groupers.

A market analysis done in 1995 indicated that the total seafood market was over 220,000mt in the main markets of Hong Kong and South China. The study forecasted this to double in every six years. In 1997, Hong Kong consumed 28,000mt of live traded fish, of which one third in volume and half in value were groupers (Hassanai and Phillips 2002). The biological characteristics of euryhaline, fast-growing, hardy and disease resistance make groupers highly suitable for aquaculture. On account of the high aquaculture potential, Bardach *et al.*,(1972) recommended them to be good candidate species for culture. Groupers can be grown in ponds, in open sea net cages, coastal enclosures and also in tanks as in the present study. They are considered a viable substitute for commercial culture in old shrimp farms in many South East Asian countries (Anon,1999).

Several species of groupers are cultured in the Asian and Pacific regions. They are cultured in cages or ponds and reach marketable size of 600-800gm in 7-8 months or 1.2 to 1.4 kg in 12-14 months. (Ruangpanit and Yashiro, 1995). Major species farmed in the Asian region are red spotted grouper (*E. akaara*), orange-spotted grouper (*E.coioides*) , brown-marbled grouper (*E.fuscoguttatus*), Malabar grouper (*E.malabaricus*), Camouflage grouper (*E.polyphekadion*), greasy grouper (*E.tauvina*), polka-dot grouper (*Cromileptes altivelis*) and coral trouts (*Plectropomus spp.*) The Chinese perch (*Siniperca chuatsi*) is farmed mostly in brackish water ponds in China. In

the Carribbean and south eastern U.S, Nassau grouper (*E.striatus*) , gag grouper *Myctoperca microlepis* and black grouper *M. bonaci* are used for farming.

6.2.Global Status

There is a lot of interest in grouper culture throughout the Asian region for income-generation and for livelihood improvement, and also as a means of reducing fishing pressure on wild grouper populations and an alternative source to cyanide-caught fish. Moreover, cultured fishes offer a means of reducing the risk of ciguatera food poisoning. Such promise of grouper culture can only be fulfilled, however, if it is operated sustainably and with human food safety in mind, as well as being based on practices that are widely accessible to coastal communities. The development of aquaculture technology for groupers will not only support an economically beneficial aquaculture sector and also contribute to reducing pressure on wild stocks. The hardiness of this fish, its tolerance to culture conditions and crowding along with its high growth rate and desired quality as food fish, make it a prime candidate for commercial culture.

In the Indo-Pacific and Middle East regions, several species of groupers are farmed in cages, ponds and tanks; groupers are cultured both in cages and ponds in most Asian countries, although cage culture is more popular than pond culture. They have been cultured in South East Asia for nearly two decades. Groupers can be easily reared, they grow quickly to a large size and provide white, tender meat; it is also a substitute crop for shrimp farming, which is still slowly recovering from decline.

Epinephelus tauvina was the first recorded species used for culture in Kuwait, Singapore and Thailand, while *E. salmoides* were cultured in Penang, Malayasia (Chua, 1978) . Many species of groupers such as estuarine grouper *E .malabaricus*, black spotted grouper *E .salmoides*, greasy grouper *E.tauvina*, red grouper *E.morio* and red spotted grouper *E.akkara* have been found to be suitable for intensive cage culture in coastal waters. However, only *E.tauvina, E.salmoides* and *E.malabaricus* are cultured on a commercial scale in South east Asia and the middle east; *E.akaara* is the species that is used for commercial culture in Japan and China. (Tseng and Foon, 1983) (**Table 12)**

In many of the South East Asian countries, galvanized iron (GI) or wood are used for constructing the cage frame. The cage is kept afloat by styroform drum, plastic carbuoy or bamboo (Kohno *et al.*, 1988). Cages are usually of 5x5x2m in Thailand (Tookwinas *et al* .,1988) .

The development of sustainable commercial grouper aquaculture has been constrained by a range of factors, but principally by the limited availability of seed (fry or fingerlings). Throughout most of the Asia-Pacific region, grouper culture is highly dependent on the capture of juvenile fish from the wild to supply seed stock for aquaculture. Although survival is sometimes lower than other cultured finfish such as seabass, grow-out culture of groupers is not technically difficult. With good husbandry and management practices like appropriate stocking densities and feeding regimes, disease prevention and treatment, good survival rates can be obtained. High quality water and sufficient depth and shading or some sort of shelter are important for most species of groupers; easy access to markets, security, pure seed availability at reasonable

price and ready supply of trash fish or high quality feed are essential requirements for this.

In Philippines grouper culture is now practiced for more than a decade. The seed of estuary grouper are collected along the shoreline and in the mouth of rivers. In Philippines cages measuring 3x3x3m are used (Kohno *et al.*, 1988). The nets used in these floating cages are polyethylene netting material with 25-50 mm diameter mesh.

The estuarine grouper *Epinephelus tauvina* is one of the most popular marine finfish species cultured in Thailand. The culture of this high valued species started some 10 years ago. Grouper fingerlings are collected from nature and grown to the marketable size of 400–800 grams in floating netcages. The culture period varies from 8–12 months. Cage culture is popularly practiced in Thailand since it is relatively easy to operate.

In Singapore grouper culture is done in floating netcages made of a simple wooden frame kept afloat by plastic buoys. A single farm is typically 1,500 m^2 anchored in a water space of 5,000 m^2. Fingerlings are mostly purchased from Thailand, Malaysia and Indonesia. Culture period is 6–8 months when the fish have attained a body weight of 600–800 grams.

Fish farmers in Malayasia have been traditionally rearing wild grouper juveniles of *E.malabaricus* and *E.tauvina* in floating netcages. The size of the netcages varies considerably although the typical sizes are 2×2×2, 3×3×3 or 5×5×5 m depending

on the number of fry available. The estimated total area suitable for finfish netcage culture is about 2,900 ha.

In China culture of groupers in floating netcages is a developing sector of the seafarming industry. The size of the netcages varies considerably according to the environmental conditions prevailing in the culture area. Common sizes are 2×2 m and 4×4 m with a depth of 2–5 m. Grouper is a highly valued species among the Chinese, however most of the national production is exported. Hongkong is a major export market.

6.3. Culture Methods

Declining catch from the oceans has made grouper and other fish culture a popular method of increasing fish production. In most South east Asian countries grouper farming is in vogue for the last two decades. Under culture system for groupers, either netcages or brackish water earthern ponds are commonly utilized. They are also cultured in large onshore tanks. Net cages are preferred in Southeast Asia and earthern or cement ponds in Taiwan. Cage culture has been practiced in many countries such as Thailand, Malaysia, Singapore, Philippines, Indonesia and Hong Kong, while pond culture has been reported to be practiced in Philippines. Typical market size is 500-1000g which can be obtained 6 to 8months of grow-out culture. Groupers are generally carnivorous and voracious feeders taking livefish, crustaceans and mollusks as food. But, it is not difficult to train grouper to feed on trash fish. For the first two months of culture, feeding rate is 10% of body weight, after which this can be reduced to 5% of body weight. (Kunguvankij *et al.*, 1986).

Grouper culture in cages :

Groupers can be cultured in net cages in sheltered coastal waters, particularly in areas where there are fishing villages. Net cages used for grouper grow-out can either be floating or stationary. Floating cages are preferred over stationary ones because they can be in areas where the tidal fluctuation is high and water more than 2m deep. Statioary cages are usually found in shallow waters with less than 1m tidal fluctuation; and they are fixed in position by wooden poles. *E.tauvina, E.salmoides* and *E. malabaricus* are cultured commercially in many South East Asian and the Middle East countries while *E.akkara* is commonly cultured in Japan and China. Tookwinas and Chrearnrid (1988) reports on the following advantages of cage culture over pond culture as:

- cage culture are usually set up in sites with better aquatic environment , and hence cages can be stocked with more fish than ponds.
- the cost of cage preparation is much more cheaper than that of pond preparation.

cage culture would not require water changing and elaborate preparation, thus making cage culture operation less costly.

Cages in three sizes are used for grow-out culture, namely 3x3x2 m^2; 4x4x2 m^2 and 5x5x2 m^2 . In the initial stocking of 9-10 cm fry, nets with mesh sizes of 2.5cm are used.

Choosing most suitable site is very important in cage culture as this would spell the success or failure. Water quality and environmental conditions of the culture site have a bearing on the stocking density. Grouper cage farm should be established in areas where water is calm ie. in a bay, behind an island or at a river mouth, in order to

avoid damage caused by strong waves or current; there should be good water quality, adequate water exchange and no predators. The site for cage culture should have good water quality, sufficient water circulation to improve the water quality that could occur due to the decomposition of waste material, which might accumulate beneath the net cage. Culture cages should be removed farther away from one another; the bottom sediment should be dredged to decrease the decomposition of waste materials. Disease outbreak should be prevented by growing strong fish which can withstand pathogens, providing fresh, high quality feed, appropriate stocking density, and suitable water quality at cage site. Cages should be protected from predators and natural hazards.

The salinity should be of 20-32ppt, although salinity more than 10ppt is also suitable. Water depth should be more than 2-3 meters. Since the usual size of culture cages is 5m× 2m and 2m deep, the tidal fluctuation should allow the water depth to be at least 2 meters at the low water of spring tide. The area should be protected from strong winds, waves and strong currents. The site should be relatively free from domestic, industrial, agricultural wastes and environmental hazards.

In a good culture site with sufficient water circulation and a high stable content of dissolved oxygen (5.54mg l^{-1}), it is estimated theoretically that 75-457 grouper fry m^2 can be stocked in a net cage to produce a harvest of 500g groupers and 40-244 fry m^2 to obtain 1200g groupers (Nabhitabhata *et al.*, 1988). Teng and Chua (1979) reported that artificial hides could be placed in the net cages whereby stocking density can be increased. Nevertheless, under normal favourable optimum conditions, the production of

groupers per cage will vary with different stocking densities. The stocking density of fry not only affect production but also appear to affect the food conversion ratio (FCR). When high stocking densities are used, the FCR of groupers is lower and vice versa.

Galvanized iron or wood are the materials commonly used for making cage frames in Thailand, Singapore and Malayasia. The cage is kept afloat by Styrofoam drum, plastic carbuoy or bamboo. In Phillippines, wooden parts are used for frame. Styrofoam drum, plastic carbuoy or bamboo are also used for supporting cage frme (Kohno *et al.*, 1988). The practical size of cage for estuary grouper is 3-11 m^2 where a stocking rate of 60 fish/ m^3 for size less than 1 kg.

Once cage has been established and fishes stocked in them, the farmer should ensure that (1) fish grow at the expected rate, (2) loss of fish due to disease and damage to nets from predators or foulers is minimized, (3) nets are regularly maintained and cleaned, (4) feeding is optimized through provision of suitable feeds for different sizes of fish at the right time of the day and at the right amount and frequency, and (5) regular grading of the stocked fish and routine checking of water quality throughout the operating period are carried out.

Stocking density for marketable fish in cage culture varies from 12-100 no/m^2, depending on water quality and environmental conditions of the culture site. Food conversion rate varies with stocking density. Trash fish is the main feed for grouper

culture in cages, given at a feeding rate of 10%, which can be reduced to 5% later. Groupers can also be trained to feed on artificial diet.

Grouper culture in ponds :

Groupers can be raised in intertidal or brackish water ponds , of size 0.5 to 1ha, which are either earthern or concrete usually of rectangular shape with depth 1.2m and a level bottom to enable easy harvest. The pond or the farm should be provided with double gate system and with separate supply and drainage to facilitate good water exchange.

To attain maximum production, the farm site must have sufficient source of sea water or brackish water; salinity and temperature of the water should be of the range 18-32ppt and 27-30 C. Water should have not less than 4ppm of dissolved oxygen; the farm also should have a reliable supply of electricity. The site must also be free from any source of industrial, agricultural and domestic pollution. The farm must also be easily accessible.

They are sometimes polycultured with tilapia in ponds. Grouper fingerlings of size 7cm or more can be stocked at 5000 per hectare. This is done one month after releasing adult tilapia (at 5000-10000 per ha) in the pond to allow them to reproduce so that the tilapia fingerlings would be food for the grouper juveniles.(Baliao *et al.*, 1998). In production of 30,000 to 40,000kg /ha; groupers are fed mostly trash fish. The culture ponds situated in the intertidal zone receive tidal flushing. Here the ponds vary in area from 0.2 to 0.3ha and have vertical dikes to hold water to a height of about 1.5m. A

continuous flow of clear water supply is maintained. If filamentous algae grow in the ponds, these are removed regularly. A pipe system is provided for daily removal of waste and excreta accumulating at the bottom. The ponds are stocked with 9-12cm long fingerlings at a density of up to 40,000/ha. The stocking density of groupers in intensive pond culture is 2-7 fish m^{-2} (Liao *et al.*, 1995). Trash fish is fed at 8% of the body weight. Under optimum management conditions, fingerlings grow to 30cm in length and 600-800gm in weight in 8 months with a survival of 80-90%.

Water quality parameters in the pond should be constantly monitored; water depth should be 0.6-1.3m, water temperature 24-31°C; salinity 20- 40 ppt and dissolved oxygen 4.9-9.3ppm**(Table 13).** Water exchange should be carried out in the pond at 50% level twice a week. Groupers take 5-7months to attain the marketable size of 400-800g.

Selective harvesting is done in many grouper culture ponds where fishes that have not reached the required size for market are released back into the pond. In case there are no immediate buyers, groupers can be kept in the net cages at 20numbers of fish/m^3, for not more than one week in these production net cages. Major constraints to the large-scale development of pond or cage culture are the shortage and uncertain supply of sufficient quantity of pure quality fingerlings from the wild.

Table. 13. ***Species of groupers cultured in the Asian Region***

Species	Common names	Countries	References
1. *Epinephelus malabaricus*	Blackspotted grouper	Thailand Philippines	Tookwinas *et al* 1988
2. *E. salmoides*	Estuarine grouper	Malayasia, Thailand	Chua and Teng(1978)
3. *E.tauvina*	Brown spotted grouper	Singapore,Kuwait	Chen *et al* 1977
4. *E. akaara*	Redspotted grouper	Japan,Hong Kong,China	Tseng and Poon(1981)
5. *E. fuscoguttatus*	Brownmarbled grouper	Indonesia,Philippines	Lim *et al* (1997)
6.E .*amblycephalus*	Whitespotted grouper	Hongkong,Philippines	Kohno *et al* 1988
7. *E. bleekeri.*	Yellowspotted grouper	Philippines,Thailand	Tookwinas 1989
8.Plectropomus leopardus	Leopard grouper	Indonesia,Singapore	Tookwinas 1989
9. *Cromileptes altivelis*	Humpback grouper	Thailand	Pakdi *et al* 1985

Table. 14. ***Water quality parameters for cage culture***

Water quality parameters	Range.
pH	7.5 -8.3
Dissolved oxygen	4.0 -8.0 mg/L
Water salinity	20-32 ppt.
Water temperature	24-32° C
Ammonia-nitrogen	less than 0.02mg/L
Hydrogen sulphide	none

Polyculture of groupers with other fish

Groupers have been cultured together with tilapia in the Philippines. A ratio of 1 grouper to 20 tilapia has proved most effective in earthern ponds. Grouper yield here is higher since they feed on tilapia fingerlings. The basic construction of polyculture pond for grouper is similar to milkfish or shrimp ponds. A suitable site with salinity higher than 10 ppt is preferred.(Tookwinas 1989). However, water management, feeding techniques, growth rate, food conversion ratio etc. have to be carefully managed, (Nammalwar *et al.*, 1998) .

Grouper is a carnivorous fish and a voracious feeder, taking live fish, crustaceans and mollusks as food. But it is not difficult to train to feed on trash fish. Feeding is at the rate of 10% of body weight initially for a few months; which can be later reduced to 5% of body weight. Since supply of trash fish may become insufficient or expensive during certain seasons and areas, fishes can also be trained on artificial diets, though without much difference in growth rate.

Although in many places , groupers varying in size from 0.5 to 1.3 kg are preferred as market-size; in Thailand preference is for fish of 0.5 to 1.0 kg. Groupers farmed in cages and ponds attain a size 0.7 to 0.8kg in eight months time. These are exported live to Hong Kong. In Taiwan many farmers have converted shrimp farms plagued by deadly disease to grouper cultivation . seawater is pumped through the ponds at 20% of pond volume per day. Grouper fingerlings are stocked at 30,000 to 40,000 pieces per pond. Harvest size varies depending on customer demand. One year grow-out

produces 0.6kg size; a 19 moth growth yielded 2.0kg size. Production was 30,000 – 40,000 kg/ha/yr with 80% survival (Aqua news1992).

6.4. Experimental culture.

A number of species belonging to the family Serranidae are highly esteemed marine foodfish particularly in the South East Asian and the Carribbean countries; many species are cultured commercially in many parts of the world. Groupers can be grown in ponds, in open sea net cages, coastal enclosures and also in tanks as in the present study. This study was thought to be of special relevance and undertaken since groupers are considered a viable substitute for commercial culture in old shrimp farms in many South East Asian countries (Anon,1999).

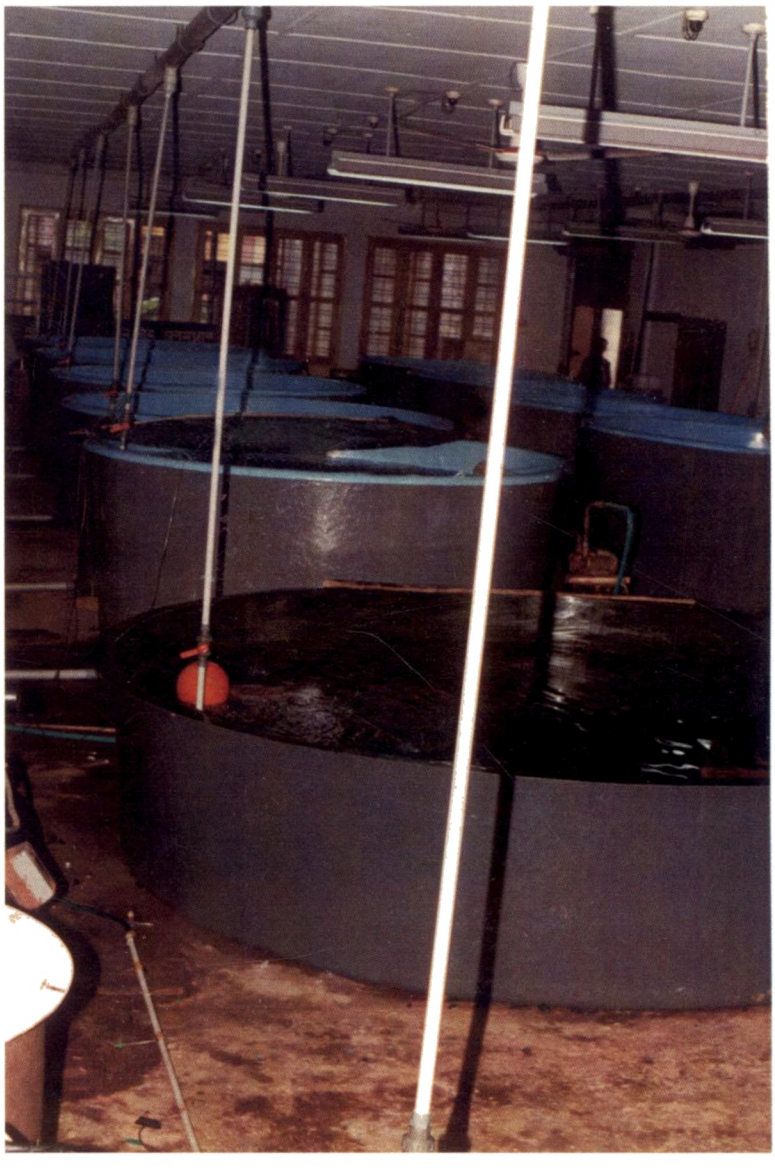
The present experiments were carried out as an attempt to culture *E.tauvina* in onshore FRP tanks of 5ton capacity with recirculating seawater system The recirculating sea water systems are especially desirable for conserving water as well as maximizing growth under conditions of water and space limitations.

6.4.1. Materials and methods

The Culture System

The present study was carried out at the Field Mariculture Laboratory of the Central Marine Fisheries Research Institute, located adjacent to the Cochin Fisheries Harbour, on the Cochin backwater system (**Plate XXI**). The culture system was housed in an area of 2500 square feet, using FRP tanks of 5000L capacity. The tanks are

PLATE XXI



Grouper culture in re-circulating sea water in indoor FRP tanks

cylindro-conical in shape, having a height of 1.25m and a diameter of 2.4 m with smooth , sea blue coloured interior. Each tank has a volume of $4m^3$. Seawater of salinity 28-32 ppt is pumped from the adjoining Mattanchery canal at the peak of high tide. The seawater after sedimentation, was treated with chlorine of strength 20 ppm, aerated well to remove all the chlorine, filtered through fine filter bags and filled into the tanks to a height of 1.1m. Two to three numbers of indegenously made biofilters were installed inside each culture tank unit. The biofilters serve the function of recirculating the water approximately 16 to 18 times a day, also discharging the filtered , ammonia free, oxygenated sea water into the system.

The in situ biofilters were made using indigenous materials such as activated charcoal, gravel, sand, oyster shells or coral pieces etc, in different layers and in various proportions. Thoroughly cleaned oyster shells are loosely packed at the bottom of an HDP bin to a height of 8 inches. A PVC pipe of 1.5 inch diameter with perforations at the bottom end to a level of 6 inches is kept at the middle of the unit to reach its bottom. A layer of charcoal of six inch thickness is spread over the layer of shells, followed by gravel and sand, each 6" thick, separated using mosquito net screens. Over this, fine sand is uniformly spread to a layer of 6" thickness. The top of the bin is covered either by a perforated lid or by a well packed sponge. The entire biofilter unit is kept immersed in sea water inside the culture tank. Water enters the unit through the perforated lid or sponge, passes through the different layers and the filtered water which is collected at the bottom of the unit is air-lifted through the central pipe and flows back into the tank. The biofilter harbours colonies of beneficial bacteria, which breaks up the ammoniacal wastes

from the metabolites of the fishes. The flow rate from the biofilter unit is regulated so as to obtain ammonia free water with optimum dissolved oxygen. The culture experiments were carried out from March 1998 to February 2000.

Throughout the experimental period, salinity of sea water in the culture tanks was maintained between 28 and 32 ppt and the temperature varied from 26.5 ^{o} c to 29 ^{o} c during peak summer. Depth of water inside the tanks was always kept to a height of 1.1m; pH was always maintained between 7 and 8 and dissolved oxygen at a saturation level of 3.5 -5 ml/L. Nitrite nitrogen and ammonia levels were also maintained at a minimum **(Fig. 13).**

Stocking of Fingerlings

Fingerlings are caught from the wild by dragnets and traps. Recent surveys conducted by the CMFRI have enabled the identification of a few grounds in the inshore waters and and the peak season for collection. Grouper seeds in the size range 60 to 200mm are collected by the fishermen operating mini shore seine and transported in well oxygenated polythene bags in sea water of salinity 32ppt, for stocking in the indoor FRP tanks at the Field Mariculture Laboratory at Fisheries Harbour, Cochin . The fingerlings were initially quarantined and sanitized by giving half an hour bath in 100 ppm formalin. Total length and weight measurements as well as the health status of the fingerlings were ascertained before stocking them in growing tanks.

Fig 13. WATER QUALITY PARAMETERS IN THE CULTURE SYSTEM

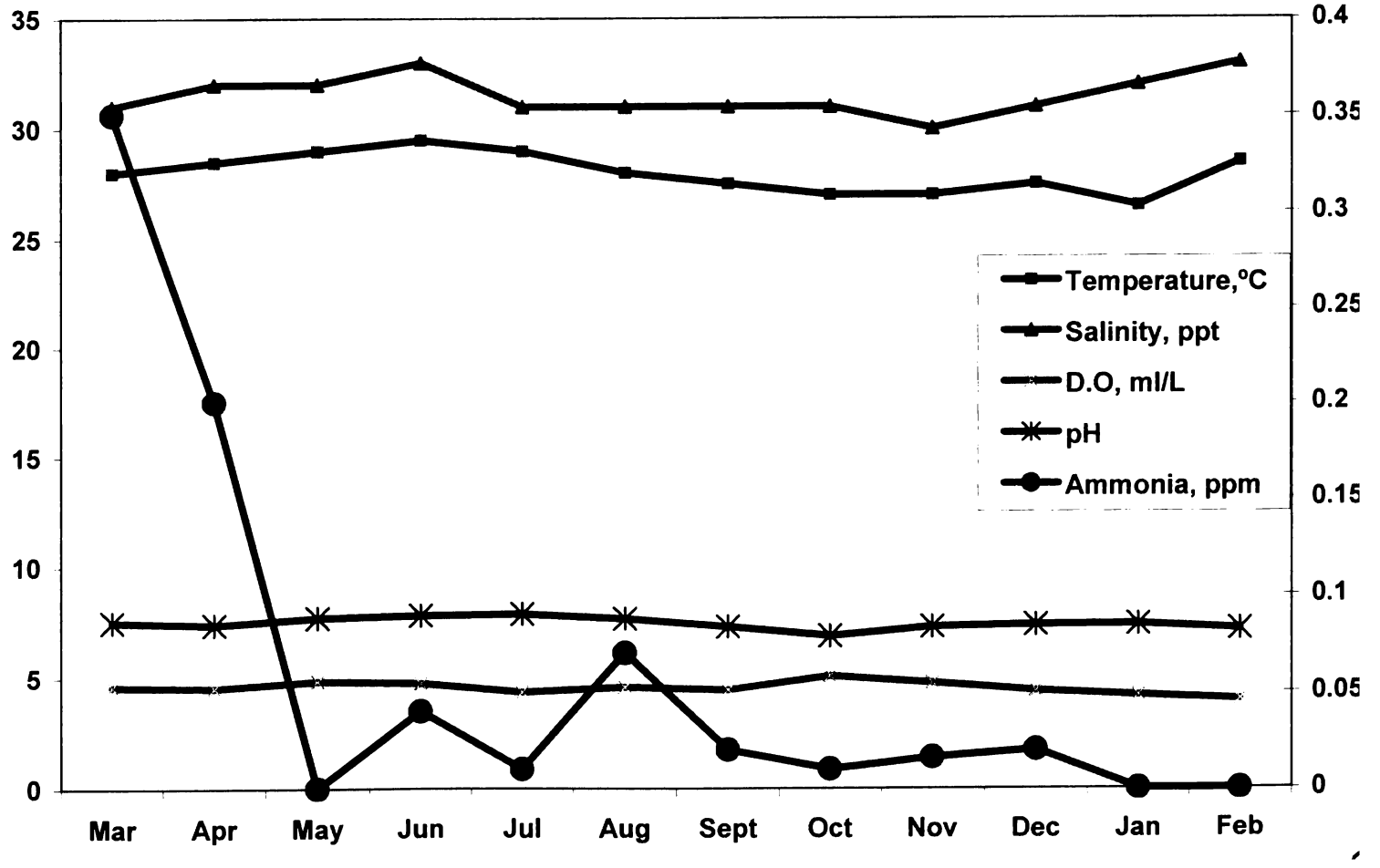


Table. 15. ***Initial stocking density, initial biomass and size of E.tauvina in grow-out experiments in FRP tanks***

Initial stocking density			Initial Size of fish	
Total no.of fish stocked per tank	Calculated stocking density (fish/m^3)	Mean Initial biomass stocked(kg/ m^3)	Mean length (cm)	Mean weight(gm)
16	4	0.182	13.5±1.2	45.5±2.3
32	8	0.398	13.3±1.4	49.8±2.2
48	12	0.750	12.6±1.4	47.4±2.1

Fingerlings were stocked according to size, since approximately uniform size reduced possibilities of cannibilism. The fingerlings were acclimatized for a few days until they fed voluntarily on chopped trash fish. The capacity of the culture tanks was calculated to be $4m^3$; this capacity was used in the calculation of initial stocking of the fingerlings. Experiments were carried out using three different stocking densities ie. $4nos/m^3$, 8 nos/m^3 and 12 nos/m^3 **(Table 15.)**; experiments using each stocking rate was replicated three times. Total length (to nearest 0.1cm) and total body weight (nearest 0.1gm) of the fish were measured once a month throughout the experiment period which lasted for 8 months.

Groupers being rock or reef dwellers in their natural habitat, were provided with a few artificial hiding places made from broken PVC, bricks or granite pieces within the tanks. Being demersal, they remain at the bottom of the tank and in the hiding places, almost sluggish, during most part of the day, moving out only at the time of feeding. Therefore, the tank bottom area is taken into consideration while determining the holding capacity of the tank.

The grouper fingerlings were fed twice a day initially. Trash fish comprising of small goatfish, sciaenids, nemipterids and small cephalopods was given as food. Feeding was to satiation, which was on an average 10% of their body weight in the early growing age. No supplementary formulated feed was given throughout this study. Faecal matter as well as uneaten feed were siphoned out daily from the culture system. Strict

Fig.14. Average growth of E.tauvina in the culture system

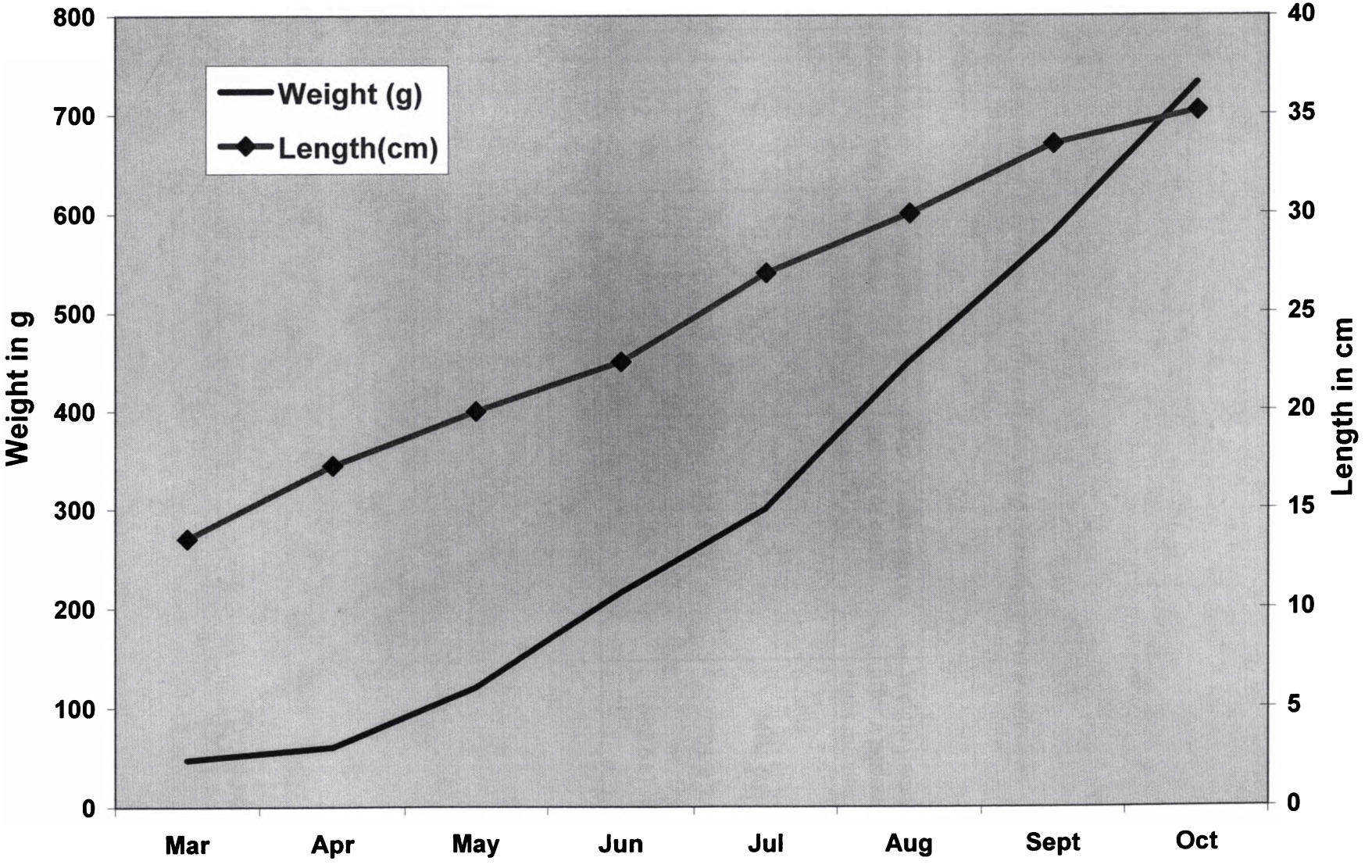


Table. 16. ***Growth of E.tauvina at different stocking densities in recirculation system***

Stocking density((fish/ m^3)	Mean Initial biomass stocked(kg/ m^3)	Net Yield after a period of eight months(kg)	Weight gain /fish after a period of eight months
4	0.182	11.840	740± 6.2
8	0.400	24.640	770± 4.3
12	0.750	32.765	682.6±1.4

measures for water quality management, sanitation and disease control were followed throughout the experimental period.

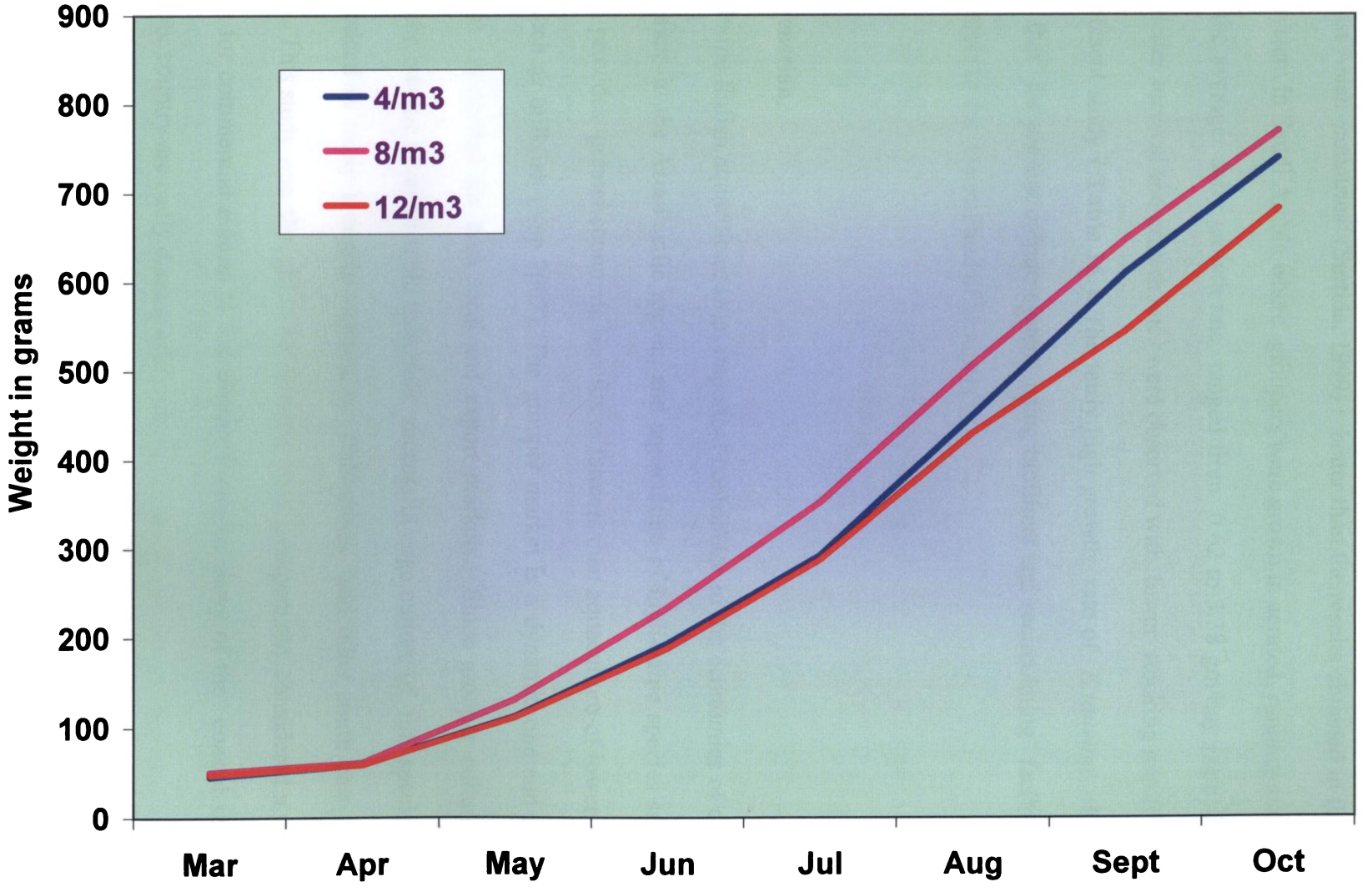
Results

Growth Rate and Production

After overcoming the initial period of stress due to transportation, the growth rate of fishes was observed to be fairly fast. Though considerable variations in growth were observed between the small and slightly larger fingerlings, the average increment in weight was observed to be 140-155 gm per month(**Fig14**). The fingerlings grew well at all the three stocking rates. At the end of seven months, the weight of the fishes was observed to range between 750 and 800gm, which is the preferred market size in Hong Kong, Singapore and other South East Asian countries. The weight gain at the second stocking rate of 8 nos/m^3 was better than the other two stocking rates of 4nos/m^3 and 12 nos/m^3 Fingerling survival ranged from 95% to 100% and was not significantly different ($P > 0.05$) among different stocking rates.

The average weight attained after seven months, in the present grow out culture system, with water quality parameters remaining constant throughout the period and feeding to satiation point, was 735gm. At the present rate of growth, the production could be estimated as 27,125 kg per hectare, within a period of eight months. The optimum stocking rate of fingerlings of mean length 135mm, determined from the present study was 35000 nos per hectare.

Fig.15. Growth of *E.tauvina* at different stocking densities



The growth of groupers in the present study, is quite encouraging. This rate of growth is comparable to that obtained in the open sea net cage culture system in the South East Asian countries (Surtida, 1999) ; better than the results obtained in the tank culture system in Saudi Arabia where groupers were grown in aerated, running sea water , where the average daily growth rate ranged from 0.62 to 3.38 gm/day (James *et al.* 1998). Better survival and growth rates were observed with lower stocking rate **(Fig.15)** in the present study **(Table 16).** The fairly high growth rate of *E.tauvina* obtained suggests that the present culture system, using biofilters and recirculating facilities, is quite feasible in the mariculture of groupers.

6.5. Discussion.

Marine finfish aquaculture is an important contributor to the economies of coastal communities in the Asia-Pacific region, and aquaculture of high-value species such as groupers provides greater economic benefits to farmers than aquaculture of lower-value species such as milkfish (Yap 2002). The grouper market is a dynamic one and added production is needed for both local and export markets. Since grouper culture can improve the income of small-scale fisherfolk, successful cage culture of this species has been practiced in many a coastal regions in Philippines and other south east Asian countries. This study was thought to be relevant since groupers are considered a viable substitute for commercial culture in old shrimp farms, as many of the coastal shrimp farms in our country are badly disease afflicted.

The growth rate obtained in the present study is comparable to that reported by Surtida (1999) in the open sea net cage culture system at the SEAFDEC and better than that reported in the tank culture system, with aerated running sea water in Saudi Arabia (James *et al.*, 1997). The growth rate obtained in the present study is much higher than the growth rates, survival and feed conversion results obtained for *E.tauvina* by various previous workers (Chua and Teng 1978, 1979,1980, Abdullah *et al.*, 1987, Ahmed *et al.*,1999,) and further confirm that this is a suitable species for aquaculture. Abdullah *et al.*, (1987) reported a growth rate of1.4-2.8g/fish/day for fish grown in raceways at different stocking densities. The present studies are comparable with the results obtained by Chou and Wong (1985) for *E.tauvina* grown for153 days in floating cages in Singapore. The growth rate obtained in the present study is higher than that reported by Hamsa and Kasim (1992) in a previous study for *E.tauvina* grown on a diet of trash fish in net cages in India. Moreover, here the fingerlings and subadults of *E.tauvina* grew and survived well at higher loading rates than are typically used in the culture of other fish species, both in tanks and floating cages.

The present study has shown that growth and survival of *E.tauvina* was comparable in tanks in recirculating sea water system and in floating net cages. Further, this study examines ways of reducing production costs and enhancing economic viability of fish farming in recirculatin systems in tanks. Here, an increase in stocking rates is made without increasing the water exchange in the culture tanks, thereby making more efficient use of sea water and reducing production costs. This study has also taken advantage of the sedentary nature of this fish, which aggregate lazily most of the time at

the bottom of the culture tanks. This behaviour results in reduced oxygen consumption rate during periods of inactivity, as indicated by McLean *et al.*(1993) for salmon being held quietly in low-velocity ponds, and for *E.coioides* in tanks (Ahmed *et al.*,2000). Fish species that swim actively in culture tanks grow better with higher water exchange rates. This study has shown that it is possible to grow the estuarine grouper *E.tauvina* on reduced water exchange rates, but with adequate oxygen provided through aeration and with water inflow through re-circulation. Culturing groupers in recirculated sea water systems at fairly high stocking densities could be of benefit in countries in which energy costs are high and water resources suitable for aquaculture are limited. However, as observed in the earlier studies by Ahmed *et al.*,(2000), excessive increasing of the stocking density of grouper may lead to undesirable crowding on the bottom surface of the tanks. As in the present study, Teng and Chua (1979) also attempted to provide artificial hides for groupers and found them to be beneficial in enhancing stocking density and overall production in net cages.

Summary

The thesis presents a comprehensive account on the reproductive physiology and breeding biology of the cultivable species of grouper *Epinephelus tauvina*(Forskaal) which is a protogynous hermaphrodite. This includes review of literature on the fisheries, biology and reproductive physiology and grouper culture.

Taxonomic status of commercially important species of groupers landed at Cochin Fisheries Harbour from commercially operated fishing craft and gear were examined, since due to the existing taxonomic confusions, there is difficulty in correctly identifying many of the epinepheline species.

Fishes used in the present study were collected from nearshore and coastal regions off Cochin bar mouth and Vypeen. The major aspects of study included investigations on the process of gametogenesis viz. oogenesis in females, sex tranformation and spermatogenesis in the protogynous males.

Morphological features of the reproductive system of both female and sex inverted male were described. The present investigation includes the classification of the ovarian maturity stages based on size, shape, colour, gonadosomatic index, texture of the ovaries, oocyte diameter, and also on microscopic structure of the ova and the morphological changes taking place within the oocyte. The process of oogenesis has been investigated on the basis of histological changes, using light microscopic

techniques. Based on the manner in which oocytes accumulated yolk and the concurrent changes in the ooplasm, cytoplasmic structure etc. 5 to 6 vitellogenic phases were described.

The Gonadosomatic index (GSI) values ranged from 0.05 in immature to 4.57 in mature gravid females. During development from immature stage to the pre-vitellogenic oocyte stage there is nearly four fold increase in the GSI, whereas the increase in GSI observed during transition from early vitellogenic to vitellogenic oocyte was around 7.5 times. Mean Gonado- Somatic Index for female *E.tauvina* ranged from 0.048 in pre-vitellogenic stage to 4.571 in mature spawning female; in sex inverted males it ranged from 0.09 to 0.131.

It was observed in the present study that in *E.tauvina*, in females, peaks in Gonado - Somatic Index and mean ova diameter synchronized with lunar cycle; further, hydrated oocytes were found during successive new-moon phases. These results suggest that gonadal development in females *E.tauvina* has lunar periodicity. In the ripe, spawning phase the hydrated and released ova measured 400-900 μm in diameter, with mode at 820μm and possessed a single oil globule. Eggs were presnt in the oviducts also.

In *E. tauvina* juveniles do not exhibit internal differentiation of sex; among fishes measuring 38-40cm in total length, the females can be distinguished by the presence of pinkish, transluscent, ribbon-like strands of gonadal tissue. Maturity stages have

been identified mainly based on the size, shape, colour and texture of the ovaries and also on microscopic structure of the ova.

The characteristic feature in the immature stage is the presence of clusters of developing oogonial cells in the active proliferation zone. The primary and secondary oogonial cells are arranged in graded manner in the ovary so that the growing secondary oogonial cells are shifted to the interior. The primary oocytes are distinguished by their basophilic cytoplasm. By mitotic division of the primary oogonial cells, the secondary ooogonial cells which are larger than the primary oogonial cells, are formed.

During secondary growth phase, lipid droplets, protein yolk globules and cortical alveoli are formed in the oocytes. These protein yolk globules appeared first in the cortical cytoplasm and later filled the entire cytoplasm.

Vitellogenic oocytes are characterised by the abundance of mature yolk. The nucleus became greatly reduced in size, lost its round shape due to the disintegration of the nuclear wall, migrated to the periphery. During late vitellogenic stages, the germinal vesicle shifts to an eccentric position in the cytoplasm and yolk fusion commences The cytoplasm and the follicle cells were wholly eosinophilic upon staining.

The hydrated and released ova measured 400-900 μm in diameter, with mode at 820μm; the yolk globules form a homogenous mass in the ooplasm, becoming more

transluscent. Lipid droplets increased in size and the single largest one at the center measured 100μm. The follicular layer became stretched due to increase in cell volume. At this stage the oocyte resumes meiotic division and are released into the ovarian cavity (ovulation) by rupturing the follicular wall.

In hormone treated fish, natural spermiation was first observed after 8 weeks and after an intake of 40.354mg/kg of fish of the hormone 17α methyl testosterone. The treated fishes became mature males after the administration of a mean accumulated dose of 50-55mg/kg/fish after 9-10 weeks. Sex inversion was induced in both in the smaller and the larger fish, with no clear relationship with fish size and age.

The gonads of fishes sampled after 30 days of hormone treatment retained the ovarian structure with a lumen and the male tissue was proliferating mainly at the periphery of the ovarian lamellae around the ovarian cavity. These also showed leftover primary oocytes in various stages of apoptosis and crypts of sperm tissue occurred in pockets among the degenerating oocytes. Yellow brown bodies were also seen.

The spermatogonia were found in large numbers along the peripheral walls of lamellae which after a period of growth multiply and formed cysts of primary spermatocytes held together by the cytoplasmic processes of the Sertolli cells. As proliferation of cells in the cysts occur, the lumen of the lobule increases.

A single spermatogonium gives rise to cysts of primary spermatocytes much smaller than the spermatogonia. Primary spermatcytes, 5μm in diamcter containcd a ccntral nucleus 2.5-3.5μm, with patches of densely staining chromatin material. Each primary spermatocytc in their the cyst undergo first meiotic division (reduction division) and gave rise to two secondary spermatocytes which are distinguishable from the primary spermatocytes by their small size (3μm) and homogenously staining nuclei. Each secondary spermatcyte underwent a second meiotic division and gave rise to two spermatids. The spermatids undergo cellular organization coupled with the formation of the tail and transform into the spermatozoa.

Female spawners measured 585.4±2.8mm to 720.2±1.8mm in total length and 3798.6± 2.4g to 6202.4± 3.4g in body weight; males were of 538.7± 4.8mm and 721.2± 3.9mm total length and body weight 3247.7±4.9g and 7098.3±2.8g respectively. Natural spawning experiments of the greasy grouper *E. tauvina* were carried out during October 1998 to July 1999 and again from October 1999 to December 2000. Successful egg fertilization was obtained by using milt from sex-inverted males, with very high fertility rate of upto 99% in the present study.

The yolked eggs were rounded and opaque, yellow in colour with diameters ranging between 0.35mm to 0.6mm. The larger transparent eggs were more advanced having diameters ranging from 0.7 to 0.9mm. The number of ripe ova or the absolute fecundity of *E tauvina* in the present wstudy was found to vary from 21,17264 to 38,98465 /fish/spawning.

Eggs were incubated at ambient temperature in 300-500 L capacity fiberglass tanks, in double filtered, u.v.treated pre-conditioned sea water of salinity 32ppt, at a stocking density of 250-300 eggs L^{-1}. Well developed embryos with eyes were observed at 17-18 hrs of incubation. Eggs hatched out in 22-23 hours at a temperature of 28-30° C. The newly hatched out yolk- sac larvae measured 1.6 to 1.7mm in length. The yolk sac on the anterior ventral side of the body occupied almost half of the total body length. The oil globule measured upto 190µm. Egg hatching rate obtained was up to 80%.

There is considerable potential for aquaculture production of groupers to replace or enhance wild-caught product, and to assist in alleviating environmental damage and overfishing. A number of species belonging to the family Serranidae are highly esteemed marine foodfish particularly in the South East Asian and the Carribbean countries. They are cultured in cages or ponds and reach marketable size of 600-800gm in 7-8 months or 1.2 to 1.4 kg in 12-14 months.

An experimental culture of *E.tauvina* was carried out in onshore FRP tanks of 5ton capacity with recirculating seawater system. The recirculating sea water systems are especially desirable for conserving water as well as maximizing growth under conditions of water and space limitations.

Experiments were carried out using three different stocking densities ie. 4nos/m^3, 8 nos/m^3 and 12 nos/m^3 . Though considerable variations in growth were observed

between the small and slightly larger fingerlings, the average increment in weight was observed to be 140-155 gm per month. The weight gain at the second stocking rate of 8 nos/m^3 was better than the other two stocking rates of 4nos/m^3 and 12 nos/m^3. Fingerling survival ranged from 95% to 100% and was not significantly different ($P > 0.05$) among different stocking rates.

The present study was thought to be of special relevance since groupers are considered a viable substitute for commercial culture in old shrimp farms, as many of the coastal shrimp farms in our country are badly disease afflicted.

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