DISCUSSION

Acceleration or control of growth can be a very useful management tool in aquaculture, especially for the hatchery production and nursery rearing of fish. Previous studies have shown that growth of finfish can be significantly enhanced by oral administration of a variety of steroid hormones (Higgs et al., 1982). Most of these studies dealt with the effects of steroid hormones on freshwater fishes, with very few exceptions (Higgs et al., 1982). The effects of selected levels of 17β-methyltestosterone (MT), diethylstilbestrol (DES), 17β-estradiol (ES) and combination of MT with thyroid hormone (T3) on the survival, growth, food utilization and body composition of the mullet Liza parsia have been examined through oral administration for the first time in the present study. The results of the present feeding experiments have shown either positive or negative influence of the steroid hormones on the survival, growth, food utilization and body composition of Liza parsia fry. The response of the fish to the different hormones and their dosages varied significantly.

Response of the fish to the dietary MT:

The present study shows that growth of Liza parsia can be accelerated by treatment with MT at optimum
levels. Further, the result suggests that the growth response of the fish to MT is dose-dependent.

Growth promotion was exhibited only by those fish fed the diets containing less than 10mgMT, with the highest anabolic effect for the fish fed on the diets containing 2mg MT. The dosages from 4 mg to 8 mg MT provided moderate growth, but the growth showed a steadily decreasing trend as dosages increased. These results suggest that the potency of the hormone declines with increasing concentration. Thus MT concentration of 10 mg in the diet produced almost similar growth to that of the control showing that the moderate dose of 10 mg of MT do not adversely influence the fish physiology. However, since there was no weight gain over the control when MT levels exceeded 10 mg/kg diet during any phase of the rearing period, it can be assumed that higher concentrations of the 17\( \alpha \) - methyltestosterone may not have any anabolic effect in the fry of this fish. In fact MT concentrations exceeding 10 mg/kg diet have a growth depressing effect in Liza parsia. These results suggest that 2 mg 17\( \alpha \) - methyltestosterone is the optimum to obtain maximum growth in this species, while the dosage of 10 mg/kg seems to be the critical level at which the normal physiology of the fish is not impaired; therefore no apparent growth
changes are seen at this concentration as compared to the control. But higher MT levels have a definite growth depressing effect on the fish fry. It is most likely that MT at supra-optimal levels either interferes with the anabolic processes of the fish or high dosages may prove toxic to the fish fry. The heavy mortality that occurred in fish groups receiving 40 to 60 mg MT can be ascribed to the above conclusion.

Results of similar magnitude have been observed, with lower concentration of MT in the diets, in the fry of many fish species. Maximum growth response has been achieved with 1 ppm MT in Oncorhynchus gorbuscha, O. kisutch and Salmo gairdneri (Fagerlund and McBride, 1977); but Yu et al. (1979) observed highest weight gains in the fry of O. kisutch with a diet containing 2.5 ppm MT, when fed to satiation. Lower dosages (1 to 3 mg/kg in the diet) of 17α-methyltestosterone are also reported to be very effective in accelerating growth in the fingerlings of many fish species: the mahseer, Tor khudree and carps, Catla catla, Labeo rohita, Cirrhinus mrigala and Cyprinus carpio (Deb and Varghese, 1988). Similarly, one year old gold-fish Carassius auratus responded with highest growth increment when received the 1 ppm MT through diets (Yamazaki, 1976). Thus the lower levels, 1 to 3
mg/kg, of this hormone promotes growth in various size groups of several fish species. However, such low levels induced only marginal weight gains in _O. nerka_ (Fagerlund and McBride, 1975) and _O. tschawytcha_ (Schreck and Fowler, 1982). In contrast, the juveniles of _Salmo gairdneri_ recorded lower growth over the control when fed on 1 to 5 mg MT (Sower et al., 1983).

In one year old gold-fish _Carassius auratus_ no apparent changes in the growth occurred at the dose of 10 mg MT while 30 mg MT diet retarded the growth (Yamazaki, 1976) as observed in _L. parsi_ fry in the present study. In contrast, moderate weight gains were noted in the fry of _O. gorbuscha_, when fed on a 10 ppm MT diet (Fagerlund and McBride, 1977), but superior growth occurred in the fry of _Cyprinus carpio_ fed on a 220 mg MT diet (Rao and Rao, 1988).

The condition factor, a measure of the relationship between weight and length is expressed for the mullet as $a = \frac{W}{L}$ where $t = 3$ in the present study (Kiron, 1989). Data obtained in the present study reveals that condition factor increases with the fish growth suggesting that growth attained is more due to the weight increment than the length increase. The steroid treatments gave higher values for
condition factor than the control fish up to the dose of 10 mg/kg, beyond this level all the fish groups recorded lower values than the fish fed on the steroid deficient diet (Table IVa, b and V). It shows that fish receiving diets containing more than 10 mg MT concentration do not add weight proportional to the normal length attained initially.

A similar response was observed in the fry of pink salmon _O. gorbuscha_ by Fagerlund and McIlhade (1977), wherein condition factor showed an increase up to the dose of 10 mg MT after 44 days. The condition factor is a sensitive indicator of changes in the diet and environment and such changes may temporarily influence the trends towards lower condition factor. Since the diet composition and environmental characteristics being almost constant for all the treatments in the present study, the variations in CF in the MT treated fish groups seems to reflect the effect of the hormone on the weight-length relationship of the fish.

Application of MT at a dosage of less than 10 mg in the diet improved the digestibility, gross conversion efficiency and FCR as against the significant reduction in the above parameters at concentrations exceeding 10 mg MT. The best
FCR, gross conversion efficiency and digestibility obtained at 2 mg MT indicates that at optimum levels of incorporation MT can improve the digestion and conversion of ingested food in L. parasia fry. This improvement is achieved either through stimulation of thyroid hormone (Overbeek and McBride, 1977; Hunt and Eales, 1979b) or by accelerating the activity of protease of the gut as observed by Lone and Matty (1981b) in the carp Cyprinus carpio. Yamazaki (1976) also found histological evidences for increase in the proteolytic activity of pancreas and intestine of O. masou treated with MT (10 ppm) for two weeks.

The FCR and GCE are response parameters which reflect upon the efficiency of conversion of the ingested food. In L. parasia both the factors were significantly influenced by the MT levels in the diet and the results are comparable to the best food conversion at 2.5 mg MT observed by Yu et al. (1979) in O. kisutch fry. Similarly, in the fry and juveniles, moderate improvement in FCE was achieved with 1 mg MT in the diet for O. kisutch (Fagerlund et al., 1979) Fagerlund and McBride, 1979) and with 2.5 ppm MT diet for rainbow trout (Simpson, 1976). Thus the improvement in digestibility and FCE observed in the present study suggest that MT augments
growth in fish by improving digestibility and conversion efficiency of the food.

Further results of the present study show that supra-optimal concentrations of MT besides lowering the digestibility of the nutrients in the feed significantly affects the metabolism of nutrients, resulting in poor FCE. It is likely that MT at hyper-dosages affects the secretion and activity of the digestive enzymes and also interferes with the metabolism of the assimilated nutrients.

In contrast to the present findings, significantly higher conversion efficiency was observed by Nirmala and Pandian (1983) in the adult fish *Channa striatus* injected with MT dosages of 5, 10, 20 and 30 mg/kg body weight. The superior performance of the above fish may be due to the larger size of the fish or the frequency of hormone administration or due to the genetic make-up of the species. Thus, it seems that the influence of MT on food conversion efficiency may be related to size, species as well as the frequency of administration.

In the present investigation, the best FCR (2.623) and PER (1.090) were recorded for the 2 mg MT treatment. These results suggest that the higher weight gains attained by *Liza*
garsia at lower MT dosages are associated with the improved digestibility, food conversion rate and protein utilization by the animal. Thus, oral administration of 17α-methyltestosterone at relatively lower dosages has significant anabolic effect in Liza parsia by improving the conversion of ingested food and protein for tissue building. Gogoi and Keshavanath (1988) also observed improved conversion efficiency rate in the fingerlings of Tor khudree at the dose of 2.5 MT (3.98) as compared to the control (5.42). Similarly, the diet containing 2 mg MT, provided better FCR in adult O.tshawytscha than the control.

The best PER recorded in the present study is in agreement with that of Yu et al (1979), who have recorded the highest value for PER in 2.5 mg MT treated O.kisutch as compared to the fish fed on the steroid deficient diet. Further, higher concentrations of MT seems to have a inhibiting effect on the protein conversion. The present findings indicate that MT levels at less than 10 mg/kg acts as a growth promoter by improving the digestibility of ingested nutrients by the fish and by increasing efficiency of conversion of the ingested food and protein. The results further suggest that optimum conversion and digestibility may be obtained at a dosage of 2 mg MT.
Body composition analysis indicates that dietary MT levels has significant influence on the protein and lipid contents. The steady increase in protein (upto 4 mg MT) and lipid (upto 10 mg MT) contents in fish associated with the increase in MT levels in the diets indicate that the rate of protein and lipid deposition is regulated by the concentration of the hormone in the diet. A concomitant drop in the moisture content was noted indicating that the protein and lipid deposition has taken place by the replacement of water from the tissues.

The present results further suggest that MT has dose dependant influence on the body composition of this fish, since the highest protein (62.8%), lipid (24.23%) and lower moisture (72.22%) levels were observed at 2 mg/kg MT diet; whereas relatively low levels of protein and lipids were found in fish fed on high MT dosages. Results of similar magnitude were recorded by Lone and Matty (1980a), when fry of common carp, Cyprinus carpio, were treated with 2.5 to 10 ppm, a sharp increase was recorded in protein and lipid contents of the fish body at both the MT levels. However, the effects of MT on body composition of salmonids appears to be different from that of Liza parsia. MT exerts only minor effects on body moisture and protein content of juvenile
salmonids. Fagerlund and McBride noted that the lower doses of MT (1 ppm) decreases the lipid level (22.39%) over the control (28.45%), but increases the protein (61.5%) as compared to the control (56.04%). In contrast, when fry of coho salmon were given 0.2 and 1 ppm MT at different temperatures, protein level increased but fat decreased at 16.5 °C, whereas an inverse trend with decrease in protein and increase in fat was noticed at 11.5 °C. Similarly, in the fry of *O. kisutch* fed on 2.5 mg MT, protein was not affected but lipid level in MT treated group (7.47%) was lower than the untreated fish (8.43%) (Yu et al., 1979). These results suggest that response of MT to deposition of body constituents is species, size and temperature dependent.

In the present study ash and moisture contents are significantly lower in the fish groups fed the 2 mg MT, which gained the highest weight. Ash and moisture levels were lower than the control upto 8 mg/kg for ash and upto 10 mg/kg for moisture. Above this concentration a steady increase was evident as the MT dose increased. The changes in the contents of ash and moisture can mainly be correlated to variation in protein and lipid contents rather than the direct effect of MT.

In the present study MT has shown severe effect on the
survival of the fish. The mortality was directly related to the concentration of MT in the diet. As a consequence, the treatment groups receiving higher dosages of MT (40 mg to 60 mg/kg) were terminated after 40 days. Further, continuation would have caused higher mortalities. The highest percentage (95%) of survival was observed in the control diet after 60 days. The lowest percentage of survival (53%) was observed in 60 mg MT treatment after 40 days as compared to the control (97%), while after 60 days the dose of 30 mg MT recorded 65% survival as against 95% in the control.

No attempt was made in this study to examine the reason for such high casualties. However, from the reduced food utilization in these groups it can be assumed that the fish have used a good proportion of the body nutrients to meet their energy demands to cope up with the stress resulted from the higher concentrations of steroid hormone. It is also possible that extreme stress might have induced morbid condition in the fish leading to eventual death. Further studies are necessary to know if there is any deleterious effect of high MT levels on the physiology of the fish.

Rao and Rao (1983) also observed relatively low survival in the fry of *Cyprinus carpio* (18.8%) given a diet with high
dosage of MT (220 ppm) as compared to the control (72.72%). In contrast, Tilapia fry, Oreochromis mossambicus did not show much difference in survival with reference to MT level of 30 ppm in the diet (89%) over the control (90%) (Macintosh, 1985).

In the present study relatively high percentage of survival was observed among fish groups fed on the control diet, and 4 mg and 2 mg MT diets. In the fry of O. gouria, S. Fagerlund and McBride (1977) observed relatively low or no mortality at the dose of 1 ppm MT in the diet. Thus the survival of fish fry is highly related to the MT dose in the diet.

Thus the results of the experiments suggest that (i) there is no beneficial effect of high MT dosages to L. parsia fry (ii) maximum growth could be achieved with relatively low MT doses (iii) 2 mg MT/kg diet is adequate enough to elicit maximum anabolic effect (iv) the response of the fish to MT is dose-dependant (v) high dosages (above 10 mg) are detrimental to growth and survival and results in overall depressing effect.

Response of the fish to dietary DES:-

Diethylstilbestrol (DES) has been widely used in
animal husbandry because of its ability to promote growth and improve food conversion efficiency without manifesting any renotrophic or hepatotrophic side effects. However, there are conflicting reports regarding action of diethylstilbestrol in fish. Ghittino (1970) reported a slight depression of body growth in rainbow trout fingerlings fed DES supplemented diets at fairly high dosages (50 - 500 mg/kg food). In contrast, Cowey et al. (1973) noted that DES, when given in low doses (1.2 mg/kg dry food) accelerated the growth rate and improve food conversion efficiency in plaice, Pleuronectes platessa, and suggested that negative growth response to oral administration of DES noted in rainbow trout by Ghittino (1970) could have been due to the use of excessive doses. In view of this argument, the growth trial presented in this study was conducted with graded levels of DES.

The results indicate that diethylstilbestrol does not promote growth or improve CF significantly when fed at even the lowest dosage of 0.3 mg/kg feed in L. parsia. DES at higher than 0.3 mg levels induced a growth depressing effect on Liza parsia. However, at low doses of 0.6 to 1.2 mg DES has been shown to accelerate growth in the fingerlings of plaice, Pleuronectes platessa, though a dose of 2.4 mg failed to promote growth (Cowey and Sargent, 1972; Cowey et al.,
Similarly, DES (4 ppm), has recorded a nonsignificant weight gain (2.8%) over the control, when fed through diet in the fingerlings of *Tor khudree* (Shyama and Keshavanath, 1988), while the same authors have recorded a negative growth for the silver carp with DES. The present results are also in agreement with the catabolic effects of DES noted in gold fish, *Carassius auratus* (Hoar, 1958), channel catfish, *Ictalurus punctatus* (Bulkley, 1972), coho salmon (Fagerlund and McBride, 1975b) and rainbow trout, *Salmo gairdneri* (Ghittino, 1970; Bulkley, 1972; Fagerlund and McBride, 1975a; Matty and Cheema, 1978). In contrast to these growth depressing results, the fingerlings of *Labeo rohita* showed superior growth when 3 mg/kg DES was fed through diet (Nanjundappa and Varghese, 1988). Thus different species of fish seems to respond differently to the same estrogen.

Likewise DES doses also did not improve the conversion efficiency, digestibility or protein efficiency ratio. With the exception of 0.3 mg dose, DES severely affected the digestibility and utilization of the food in *Liza parsia* (Table VI). Similarly, no significant difference was observed in FCE and protein conversion efficiency in coho salmon *O. kisutch* administered with 2.5 mg DES (Yu *et al.*, 1979). In contrast, Cowey *et al.*, (1973) noticed improvement
in food efficiency when *Pleuronectes platessa* was fed on a diet containing DES doses of 0.6 and 1.2 mg/kg dry food). Nanjundappa and Varghese (1988) also observed improved food conversion efficiency when fingerlings of *Labeo rohita* received 1 mg DES.

Proximate composition data indicate that body protein and lipids were increasingly catabolized for energy production rather than tissue building with the progressive increase in DES levels in the diets. Water and ash levels in the tissues steadily increased as a result of the protein and lipid catabolism. Yu et al. (1979) did not find any significant difference in protein and ash contents, though lower values for lipid were recorded in *O.kisutch* receiving 2.5 mg DES in the diet. Similarly, significant differences were not observed for moisture and total nitrogen in the juvenile rainbow trout when fed on diets containing 1.2 mg/kg DES (Matty and Cheema, 1978).

From the gains in weight and body protein at 0.3 mg over the control it can be inferred that DES levels less than 0.3 mg/kg may have some growth promoting effect in *L. paresia* fry.

In conclusion, from the response data obtained during
this study, it can be inferred that there is no advantage in adding DES to the diets of \textit{L. parsia} fry. Further, the future of DES has been called into question because it is carcinogenic (Anonymous, 1972 cited by Cowey \textit{et al.}, 1973). Clearly, the metabolism of DES and the rate at which it is removed from the carcass during feeding on a diet free of DES will require careful investigation before its use in production diets may be contemplated.

\textbf{Response of the fish to dietary 17}^\text{-} \text{ Estradiol(ES)}:-

The estrogen 17$^\beta$-estradiol (ES) proved to be a growth promoter for \textit{L. parsia} as evidenced by the response attained in the present study. However, only moderate increase in growth (11.1\%) was observed with 2 mg ES as compared to the fish fed on the hormone deficient control diet. Specific growth rate and condition factor are also found to be enhanced (Table VII) in this group. The increase in condition factor (1.180) at 2 mg/kg estradiol diet over the control (1.098) shows that the increase in growth is attained through weight increment rather than increase in length. Probably, this concentration may cause enhanced food utilisation to account for the growth increment.

The growth promotion in \textit{L. parsia} is in agreement with
the observation of Yu et al. (1979), who noticed about 15% weight gain over the control in coho salmon, *O. kisutch* on a dry diet supplemented with 2.5 mg estradiol. However, the same species (*O. kisutch*) gave only 5.5% weight gain over control, when the dose of this hormone was increased to 10 mg/kg (Fagerlund and McBride, 1975; Donaldson et al., 1979).

The fry of the common carp seems to require relatively higher concentration of this hormone to produce weight gain compared to the present study. Rao and Rao (1983) observed an average weight gain of 50.98% in the diets with 200 mg estradiol per kg; whereas 120 mg/kg estradiol had a growth depressing effect (Jensen and Shelton, 1979). On the other hand Yamazaki, (1976) could not come to any conclusion when *Carrassius auratus* were treated with 1 - 10 mg/kg of this steroid. Thus the response of fish species to the estrogen, estradiol seems to be dose as well as species dependant.

Relatively better values for digestibility, food conversion rate, gross conversion efficiency and protein efficiency ratio were found for fish groups receiving 1 and 2 mg estradiol in the diet (Table VII). But, the highest feed conversion efficiency (28.1%) and PER (0.809) were recorded
for fish given 2 mg estradiol in the diet. It indicates that 2 mg estradiol is the optimum dietary level required for maximum growth in *Liza* *parsia*. Response of similar magnitude for FCE and PER were observed in coho salmon (*O.kisutch*) fed on diet containing 2.5 mg/kg estradiol (Yu et al., 1979). This hormone functions as an anabolic agent in *Liza* *parsia* by improving the digestibility and conversion of food and protein into tissue.

The results of proximate analysis are presented in Table (VII). ES did not have much influence on the body composition of *Liza* *parsia*. However, slightly higher values for protein and lipid as well as lower levels of moisture and ash percentages were observed in the fish treated with 1 and 2 mg estradiol. Estradiol level exceeding 4 mg/kg produced fish with relatively lower protein and lipid than the control fish suggesting catabolic effect of higher levels of this steroid on body nutrients in *L. parsia*. In *O.kisutch*, estradiol (2.5 mg/kg) diet did not induce any significant change in body composition as compared to fish receiving a steroid deficient diet (Yu et al., 1979).

Results of this experiment indicates that estradiol supplementation at the dose of 2 mg/kg diet can be beneficial, in that *Liza* *parsia* could be raised to desired
size in a shorter period of time, with relatively less food and better feed efficiency.

Response of the fish to thyroid hormone (T3) and MT levels and their combinations:

A) Thyroid hormone (T3)

Oral administration of thyroid hormone (T3) caused significant weight gain in Liza parsia fry. Besides, T3 hormone along with MT serves as a growth promoter in this fish. Oral administration of T3 induced significant improvements in weight gain, conversion efficiency, as well as protein and lipid contents of Liza parsia over the control.

Data shows that relatively low doses (3 and 6 mg) of T3 improves growth and condition factor in Liza parsia over the control (Table VIII). The anabolic effect may be caused by several mechanisms, operating individually or synergistically: (i) through the ability of thyroid hormone to potentiate the effects of other anabolic hormones, most notably growth hormones (Donaldson et al., 1979; Eales, 1979), (ii) through stimulation of DNA-dependent RNA synthesis and subsequent protein synthesis (Higgs et al., 1982), (iii) T3 may potentiate appetite and or food
utilization (Markert et al., 1977). Anabolic effect of T3 has also been reported in *O. kisutch* (Fagerlund et al., 1980) and *Salmo gairdneri* (Eales, 1979 b).

Comparing the different dosages used in the present study, superior growth has been achieved with 3 ppm T3. The dose of 9 mg T3/kg diet seems to have a depressing effect on growth and feed utilization in *L. parsi*. Juveniles of *Salmo salar*, however, seems to require higher dosages (20 mg T3) for the optimum growth (Saunders and Henderson, 1980, cited by Higgs et al., 1982). These results suggest that the anabolic effect of T3 in fish is size as well as species oriented (Higgs et al., 1982). Besides the hormone dosages, many other factors seems to influence efficiency of T3, i.e. route of administration and nutritional status of the fish etc. In the present study, the composition of the diets used was maintained quite uniform and hence the response attained relates to that induced by T3 incorporation alone.

While the growth, FCR and PER were better for the 6 mg T3 diet the protein and lipid levels were higher for fish receiving the 3 mg T3 diet suggesting that maximum protein synthesis and lipid deposition occurs at relatively lower levels of T3 and that T3 significantly influences the protein and lipid deposition in *Liza parsi* fry.
The results of gross conversion and protein conversion efficiencies and digestibility coefficient suggest that growth promotion in *Liza parsia* is attained through the positive influence of T3 in improving utilization of food and protein. As compared to the GCE and FCR obtained at 3 mg T3 in *L. parsia*, Fagerlund *et al.* (1979), obtained better FCE and PER at the dose of 2mg T3 in the fish *O. kisutch*. Results of similar magnitude for FCE and PER were recorded in *O. tschawytscha* (Higgs *et al.*, 1982).

The dose of 9 mg/kg resulted in poor feed utilization suggesting that T3 at supra-optimal levels affect appetite or food utilization. Similarly, Fagerlund *et al.* (1979) noted that the high dose (25 ppm) of this hormone results in lower food and protein conversion efficiencies in *O. kisutch* but in *Liza parsia* the food and protein utilization is affected at relatively low dosages of T3. Diet composition (Fagerlund *et al.*, 1979; Higgs *et al.*, 1982) and prevailing hormonal milieu (Fagerlund *et al.*, 1980) are factors that are suspected to affect the potency of T3 in enhancing food and (or) protein utilization.

The fish on the 6 mg T3/kg diet were found to be very healthy and consequently high survival rate (97%) was
recorded; whereas the fish groups receiving 9 mg/kg T3 recorded low percentage of survival (83%) suggesting that the fish may be under physiological stress induced by high level of T3 in the diets.

In mammals thyroid hormones play an important role in the regulation of appetite, digestion, nutrient absorption, protein anabolism and catabolism and in non-protein energy deposition and mobilization (Felber, 1977). There is also evidence for thyroidal involvement in all these physiological processes at least in some fish species (Donaldson et al., 1979). In L. parsia digestibility of the ingested food has been affected by the T3 level in the diet with T3 levels exceeding 6 mg inducing a significant decrease in digestibility. These results seem to suggest that high T3 levels may interfere with the activity of some digestive enzymes. Further research is necessary to confirm this speculation. On the other hand, improvement in the utilization of food and protein for growth as evident in Liza parsia is commonly observed (Higgs et al., 1982).

In the present study smaller gains for protein (61.5%) and lipid (23.83%) were observed in fish treated with 3 mg T3 diet as compared to the control (Table VIII). The gains
recorded for protein may be caused by the action of T3 on RNA synthesis and thereby protein or enzyme biosynthesis in the fish body (Higgs et al., 1982). In juveniles of O. kisutch the diet incorporated with 4 ppm T3 showed a slight improvement in protein of the fish content (62.5%) as compared to control (60.5%) (Fagerlund et al.; 1980). However, the lipid and moisture contents were lower in this treatment (4 ppm) than the control diet. In contrast, significantly lower values for body protein were observed in Salmo gairdneri when given T3 dosages (0-25 ppm) through diets (Higgs et al., 1982). The present results confirm the observations that deposition or mobilization of protein and lipid may depend upon hormone dose (Narayan Singh and Eales, 1975). However, circulating levels of other hormones which influence body energy reserves in fish, such as growth hormone (Donaldson et al., 1979) and prolactin (Meier, 1970) may also interact with thyroid hormones to determine the response direction.

B) Hormone combinations:

For the first time the combinations of a steroid hormone (MT) and a thyroid hormone (T3) have been tested on Liza parsia at various concentrations. To the author's knowledge there is no other report relating to the combination of MT
and T3 to test fish growth, although several other combinations have been evaluated. However, Higgs et al. (1977) have studied the effect of combination of T4, bGH and MT as well as T4 + MT on coho salmon.

Results of the present study suggest that growth enhancement also can be achieved by the combination of MT and T3 at optimum doses over the control. For instance, maximum growth in *Liza parsia* occurred, when a diet with a combination of 9 mg T3 + 2 mg MT was fed. The same fish group gave the second best values for specific growth rate and condition factor amongst all the groups.

In the present study the 9 mg T3 + 2 mg MT/kg diet combination provided the highest growth up to 30 days probably due to the synergistic effect. This combination would have created an internal hormonal milieu most conducive to rapid growth of the *Liza parsia*. Hormone combination probably stimulate growth by improving appetite and (or) food conversion efficiency as has been shown for bGH (Markert et al., 1977) and MT (Higgs et al., 1977). The steroid (MT) would have contributed to the growth promotion by stimulating deposition of protein and lipid, while T3 may promote feed conversion ability of the fish (Higgs et al., 1982). From
studies with mammals it is known that the hormones which have the greatest influence on cartilage and bone development are the thyroid hormone, androgens and growth hormones, and the thyroid hormones and sex steroids are more active on the process of calcification and ossification (Rapport, 1975). This may also be the case in fish. Some hormone such as thyroid hormone influence somatomedin activity in mammals, which may mainly regulate chondrogenesis and hence affect the rate of growth (Gaspard et al., 1975). A similar situation may exist in fish.

The fish groups fed on the dosage of 3 mg T3 + 2 mg MT per kg and 3 mg T3 + 4 mg MT per kg gave lower growth increment over the control, suggesting that these combinations are not appropriate to promote the anabolic effect in *Liza* *parsia*. It is likely that the anabolic effect of the combination of this hormone may also be dose-dependent and that specific ratios of hormone levels may also be necessary to induce a growth promoting effect.

The increase in condition factor, noted for 9 mg T3 + 2 mg MT per kg fish group indicate that this proportion of hormones induces greater growth in weight than length in *Liza* *parsia*.
Although the combination of 9 mg T3 + 2 mg MT per kg gave the second best growth promotion, the survival rate (83%) was low as compared to the control as well as the fish groups receiving individual hormones [6 mg T3 per kg (97%), 2 mg MT per kg (98%) and 4 mg MT per kg (93%)] suggesting that as far as survival is concerned, the individual treatments are better than the hormone combinations for the fry of *Liza paria*.

Combinations of T3 and MT treated fish groups recorded better food utilisation than that of control, except for the fish groups receiving 3 mg T3 + 2 mg MT, and 3 mg T3 + 4 mg MT. The best FCE and Da recorded in fish fed on 9 mg T3 + 2 mg MT indicates improved assimilation of the ingested food at this level of combination, resulting in improved weight gains, food conversion and protein utilisation in the fish.

Results obtained (Table VIII) shows that, the combination of MT and T3 changes the proximate composition of *Liza paria*. Most of the hormone treated fish had greater levels of protein and lipid and lower moisture and ash percentage than the controls. The highest percentage of lipid (23.76%) and protein (64.43%) were noted in fish fed on 9 mg T3 + 2 mg MT. The lower contents of moisture and ash in the fish group receiving 9 mg T3 + 2 mg MT over control gives an
evidentary proof in this study that, the combination of T3 and MT at this level has stimulated the accumulation of more protein and lipid in *Liza parsia*.

The results suggest that hormones combination can be used to manipulate the proximate composition, food conversion efficiency and condition factor in *Liza parsia*. From the response of the fish to the various hormones and their combinations 2 mg/kg 17α-methyltestosterone seems to be the best for the maximum growth promotion if the experimental duration is longer, while for shorter duration the dose of 9 mg T3 + 2 mg MT per kg would be ideal.

In *Liza parsia* the androgen MT seems to be more suitable than the combination of T3 + MT.

Response of the fish to MT at different dietary levels of protein:-

Since 17α-methyltestosterone was found to be the most potent growth promoter in *Liza parsia* fry an experiment was conducted to ascertain whether the growth promoting effect could be achieved by a reduction in protein level by incorporating MT. Secondly, to the author’s knowledge, so far, only in two species the effects of androgens on the
protein sparing action has been investigated (Matty and Cheema, 1978; Lone and Matty, 1982; Fagerlund et al., 1983). Of these, only Fagerlund et al. (1983) evaluated the protein sparing effect of MT on D. kisutch, while the other study was conducted with another androgen, ethylestrenol, on Cyprinus carpio (Matty and Cheema, 1978; Lone and Matty, 1982).

In the present study irrespective of the MT levels (1, 2 and 3 mg/kg) all the diets containing 30% protein produced superior weight gain and specific growth rate in the fish as compared to the 35% protein diet. The percent weight gain and SGR suggest that optimum dose of MT for Liza parsia is 2 mg/kg at the dietary protein level of 30%. At this combination MT proved to be most effective as a growth promoter. Likewise, 35% protein + 2 mg MT/kg also produce higher weight gain as compared to other levels of MT in 35% protein diets.

However, the minimum protein level in the diet necessary to produce maximum growth in Liza parsia fry at the dose of 2 mg/kg MT seems to be about 30% dietary protein. This is significantly lower than the protein requirement of 40% recommended for the fry of this species (Kiron, 1989) using purified diets. Thus, the inclusion of the steroid hormone,
MT, improves protein utilization significantly for promoting optimum growth.

However, Fagerlund *et al.* (1983) have observed higher weight gain in *O. kisutch* fed on diet containing higher protein level (51%) than lower protein levels (35%), though both diets had 1 mg MT/kg. The present results confirm the earlier report made on rainbow trout fry by Matty and Cheema (1978), that fish receiving a lower protein diet (35%) with 3.5 mg/kg ethylestrenol produce significantly higher weights than diets with 40 and 45% protein. The relative poor growth in 35% and 25% dietary protein levels in the present study indicates that in the presence of an anabolic agent like MT in the diets, dietary protein levels above 30% is poorly utilized for the growth. Probably some amount of protein is catabolized at this dose.

Decreasing the dietary protein content up to 30% containing MT (2 mg/kg) increased the growth in relative to length. This resulted in significant increase of the condition factor of *Liza parsia*. In contrast, ratio of weight to length was found to be decreasing gradually with time when *O. kisutch* fed on diet containing dietary levels of protein and lipid with the inclusion of 1 ppm MT (Fagerlund *et al.*, 1983).
Besides acceleration of growth, another factor which is of considerable relevance to fish farming is the efficiency with which fish can convert food into flesh. Present findings show that the fish groups had improved digestibility, conversion efficiency, protein efficiency ratio and food conversion rate at the dose of 30% protein + 2 mg MT. The enhancement in conversion efficiency may be due to the improved intestinal absorption as reported by Habibi and Ince (1983) and Habibi et al. (1983). Therefore, assimilation and conversion of nutrients notably proteins seems to be increased in the fish body at this dose. These findings support the earlier observations of Fagerlund et al. (1983) who found PER to be inversely related to the dietary protein levels when O. kisutch fry were fed on a lower dietary protein diet with supplementation of 1 ppm MT. Thus, the results suggest that MT improves protein utilization in fish when protein content of the diets are relatively lower than the normal levels.

The results (Table IX) of body composition indicates that the changes in the protein, lipid, moisture and ash are only marginal. However, the fish fed on the diet containing 30% protein with 1 mg, 2 mg and 3 mg/kg MT recorded slightly higher protein and lipid levels than the other diets of different MT concentrations.
The highest protein and lipid content in the fish groups receiving 30% protein + 2 mg MT/kg suggests that in this treatment MT had its highest anabolic effect. Unlike the present findings the MT supplemented low protein diet (35%) did not affect the body composition of *O. kisutch* (Fagerlund et al., 1983). The increase in body contents of protein in *Liza parsia* might have occurred due to the increased incorporation of amino acids into proteins, as a result of the stimulation of RNA synthesis by MT, as observed by Matty and Cheema (1978) in *Cyprinus carpio*.

Survival rate was above 90% in most of the groups containing 35% and 30% protein with different concentrations of MT. Similarly, the fish groups receiving 25% protein + 1 mg MT/kg also gave 90% survival rate (Table IX), suggesting that the survival of animals will not be adversely affected by the lowering of dietary protein levels in MT supplemented diets.

Results of the present study suggest that *Liza parsia* fry may be reared on a 30% protein diet by incorporating 2 mg MT for achieving maximum growth, food and protein utilization. The manipulation of diet composition and MT content provides the fish culturist a means to control growth
and thus helps in attaining the optimum size with savings in cost of feed.

Response of the ovary of fish to injections of MT and Estrone:

(i) MT Injections

The ovaries were observed at the termination of experiment, which initially were found to be immature (in oogonial stage). Ovaries of all the three groups of MT treated fish appeared normal except that some ova were slightly larger in size at all the treatments. All the MT-injected fish had ovaries, which were in the central nucleolus stage as against the oogonia of the control fish indicating that administration of MT triggers the initiation of vitellogenesis. However, all the ovaries were in similar state of development and none of the doses produced further development, after the central nucleolus stage. These results suggest that administration of MT stimulates vitellogenesis, but inhibits further ovarian development within the dosages employed in the study.

Fagerlund and McBride (1975) obtained similar results. After injecting MT doses, 10 - 50 mg, the ovary of O. kisutch
was found to be unaffected. However, when this steroid level increased to 100-500 mg/kg marked degeneration occurred in the ovary suggesting that high dosages would deleteriously affect the development of ovary.

(ii) Estrone Injections

In the present study some changes were seen in the ovaries of the estrone treated fish. The ovary of the controlled fish groups were in the oogonia stage. In contrast to the ovaries of the fish treated with 1 mg and 2 mg/kg estrone which were in the chromatin nucleolus stage, the fish receiving the injections of 3 mg/kg estrone were in perinucleolus stage of development. Thus injection of estrone stimulated the development of the ovary towards maturation with the progressive increase in dosages from 1 to 3 mg/kg of fish producing a steady advancement towards the maturation of the fish. Past experiments gave contradictory results when estrone was injected to finfish species. Estrone injection causes proliferation of oogonia in the ovary of minnow, Phoxinus laevis (Bullough, 1942), gudogeon Hypselectris galli (Mackay, 1973), while the 45 rat unit of estrone/week did not affect the ovaries of L. reticulatus (Berkowitz, 1938) at 3,00,000 IU unit inhibited the degeneration of Labistes reticulatus ovary (Svardson, 1943).