5. DISCUSSION

5.1 Studies on annual variations in thyroid gland, ovary and liver of H. fossilis

Majority of the evidences showing cyclical activity of the fish thyroid is derived from histological and cytochemical studies conducted during different seasons or during metamorphosis. However, qualitative studies using histological methods in teleostean thyroid were started long time back when the activity was based on the size and number of epithelial cells, height of epithelial cells amount of colloid in it (Buchman, 1940; Barington and Matty, 1954). In the present study evaluation of thyroid activity in H. fossilis has been performed according to the method of Fortune (1955) for want of laboratory facilities for radioiodine uptake or radioimmunoassay techniques.

High TAI, increased vacuolization and reduced colloid contents have been taken as reflections of high activity of the gland. Using such parameters it is evident that in H. fossilis thyroid glands attains the maximum activity during July-August and minimum during December-January.

Both photoperiod and temperature had similar seasonal variations throughout the year in being maximum during June and minimum during January. It is, thus, evident that longer photoperiod is characterised by an increase of atmospheric temperature. The temperature started declining from July with the commencement of the monsoon and heavy rain fall. Thyroid activity and natural photoperiod as well as temperature were well correlated with each other in this species which seems that environmental factors like temperature, photoperiod and rainfall provide necessary stimuli for thyroid stimulation. Similar correlation between thyroid activity by measuring either epithelial cell height or radioiodine uptake or plasma thyroid hormone levels and natural photoperiod and temperature were also recorded in several fish species (Swift, 1960; Singh, 1968; Singh and Sathyanesan, 1968; White and Handersen, 1977; Chakraborti and Bhattacharya, 1984; Cyr et al., 1988a; Srivastava, 1990).

Ovarian growth coincided with an increase in temperature and photoperiod throughout the annual reproductive cycle of this species. The temperature started declining from July while GSI from August. It seems that during this period (spawning period) fish awaits the rainfall which lowers the temperature and initiates the ovulation and spawning (Singh and Singh, 1990). Similar observations were made where photoperiod and temperature induced ovarian development and maturation in fish (deVlaming,1972; Sunderaraj and Vasal, 1976; Osborn et al.,1978; Mohan et al.,1988; Bhat and Dutt, 1989). It is concluded that in H. fossilis ovarian

recrudescence appear to be directly related to increased photoperiod and temperature while regression occurs during the period when there is decrease photoperiod and temperature.

In teleosts, as in other oviparous vertebrates, yolk proteins appear to be synthesised outside the oocytes (heterosynthetic) in liver (reviewed by Fostier et al.,1983). In addition to this heterosynthetic process, and intraovarian origin of yolk proteins (autosynthetic) has also been suggested by histological studies (Verma et al.,1989). The size of the ovary starts increasing from preparatory to the spawning phase due to the accumulation of large numbers of oocytes and deposition of york materials, from extraoocytic or intraoocytic or both, in the ova. Increasing levels of oocytes, ovarian protein and GSI values during these periods may be an indication of this fact.

Protein contents of ovary and liver increased progressively from preparatory to spawning phase and reached to their peak values in July. This might be due to increased synthesis and/or decreased degradation of protein and/or increased amount of enzymes required for protein synthesis. During the present study lipid estimation could not be done and therefore, above suggestion is based on the analogy of other reports. This change possibly suggests that during such periods liver acts as store house of nitrogenous products. Similar findings have been recorded in many fish species (Idler and Bitners, 1960; Jafari and Khawaja, 1968; Bano, 1977; Sower and Shreck, 1982; Verma et al.,1985, 1989; Lal and

Srivastava, 1989; Singh, 1991). However, peak levels of protein were found in ovary during spawning and liver during just before spawning in Esox lucius (Medford and Mackay, 1978) and Salmo gairdneri (van Boheman et al.,1981).

Early post-spawning phase is characterised by a flaccid and regressed ovary, which is reduced in volume while late post-spawning phase by smallest ovary containing only nests of oogonia hence a low GSI was recorded. Ovarian decline of oocyte diameter and ovarian protein in post-spawning period of fish may be due to the discharge of mature ova from the ovary to outside and/or less demand of protein. Liver protein levels were also decreased with decreasing ovarian protein levels. Similar observations were reported by Medford and Mackay (1978), van Boheman et al. (1981), Verma et al.,(1985), Lal and Srivastava (1989), Singh and Singh (1990). It is apparant from the results that increase and decrease of protein levels in ovary and liver are well correlated with each other throughout the year in this species.

Liver glycogen and HSI values were found to be minimum during spawning period. Large amount of energy is required by the fish during gonadal development (Love, 1970; Mackinnon, 1972). Hepatic glycogen probably represents the energy source to meet out the metabolic needs encountered in ovarian growth and spawning (Petersen and Emmersen, 1977; Lal and Srivastava, 1989). The glycogen constituents of the liver is also used as raw materials during the formation of yolk proteins (Verma et al.,1989). The

decrease in liver glycogen contents in spawning phase has been suggested to be due to steroid hormones released during this period (Dasmahaptra and Medda, 1982). Since liver is one of the vital organ for different metabolic activities, the observed gradual diminition of glycogen content during gonadal maturation in H. fossilis may be because of glycogenolysis to meet high demand of energy required for gonadal maturation and/or used as raw materials during vitellogenesis. Decreased level of HSI in the spawning period may be a reflection of this fact. Increase in ovarian glycogen during spawning phase suggests increased synthesis of glycogen in ovary and/or transfer of glycogen to ovary. The observed increased in ovarian glycogen at a time when liver glycogen was found to decrease probably suggests that there is transfer of glycogen reserves from liver to ovary during spawning period. It seems that ovarian development might be through this pathway atleast for the energy purpose.

It is recorded here that in H. fossilis ovarian moisture percentage were maximum during the months of post- spawning when both glycogen and protein were minimum. Presumably depleted glycogen and protein contents were replaced by water during this phase. In liver too moisture value was found to increase during gonadal maturity period and maximum moisture percentage coincided with spawning phase. Decreased water percentage in ovary during spawning phase may seem to indicate excess deposition of dry matter. Similar findings have been noticed by Jafari and Khawaja (1968) in O. punctatus , Lal and Srivastava (1989) in

H. fossilis and Piska and Prasad (1991) in S. bacaila. Increased level of water content in liver during breeding season may be due to diversion of dry matter towards gonadal development and maturity.

It is evident from the results that thyroid attains maximum activity during July-August when oocyte diameter, ovarian protein, GSI and ovarian glycogen were maximum. Once the spawning is initiated thyroid activity starts declining reaching its minimum level in December-January. The thyroid activity exhibited positive correlation with these parameters throughout the year. Highest level of epithelial cell height (Buchman, 1940; Barrington and Matty, 1954) and thyroid hormones production (White and Handersen,1977; Chakraborti and Bhattacharya, 1984) has been recorded during spawning phase. Positive correlation between thyroid activity and ovarian changes like ovarian weight, ova diameter and GSI have been reported in C. batrachus (Dubey and Choubey, 1988) and H. fossilis (Lal and Srivastava,1990a). From the ongoing discussion of this investigation it seems that a close relation exists between thyroid and ovarian dynamics. Similar parallel changes between thyroid and liver protein and moisture contents are also evident during the different months of the year. However, hepatic glycogen and HSI values were inversely related with TAI. Similar observation of negative correlation between TAI and HSI has been published by Lal and Srivastava (1990a).

In conclusion, it may be opined that the observed association between thyroid and ovarian maturation in H.fossilis might be through the liver and environmental factors.

5.2 Effects of thyroid hormone on ovary and liver of H.fossilis

The results from the annual study of thyroid (see Section 4.1) seem to indicate that thyroid hormones are probably necessary for ovarian growth and maturation in this species. The involvement of thyroid in gonadal maturation of teleosts has been largely inferred from the effects of thyroidectomy (using goiterogens or massive doses of radioiodine) and from observations of cycle in thyroid activity coinciding with gonadal maturation (reviewed by Hoar, 1965; Sage, 1973; Eales, 1979; Nagahama, 1983; Cyr et al., 1988a). In the present investigation T_4 and thiourea were used to modify the thyroidal status to assess the impact of thyroid on the ovarian development. Chamber (1953) observed that thiourea treatment caused inhibition of gonadal maturation in F. heteroclitus. It is well known that thiourea is a metabolic inhibitor and therefore, its effects can not be judged as antithyroid drug effect alone. Looking into these facts, we have used minimum quantity of thiourea in the present study.

Thyroid hormones play an important role in the regulation of general metabolism in mammals, if this is true in fish, they might serve to increase the availability of nutrients, metabolites and yolk precursors for ovarian development. In addition, thyroid hormones may directly influence ovarian metabolism, perhaps

increasing sensitivity to gonadotropic stimulation. Although a role of the thyroid in the control of general metabolic rates has not been conclusively demonstrated in teleosts, regulatory effects of thyroid hormones on protein metabolism have been reported (Narayansingh and Eales, 1975; Medda and Ray, 1979; Ghosh and Medda, 1982; Matty et al.,1982; Dasgupta and Medda, 1990). Our observations (see Section 4.2.1) that T_4 treatment increased the protein content of ovary and liver in intact fish lend further support to the above views. This is confirmed by the fact that thiourea produced opposite effects.

It has been suggested that under the influence of gonadotropin, the synthesised protein accumulate in the ovary (Sundararaj et al.,1982; Breton and Guimard, 1983). Such an accumulation of protein in ovary might account for increased ova diameter and GSI values as reported in this investigation. In general, thyroid hormones are associated with protein anabolism in that they enhance growth under appropriate conditions, modify synthesis of haemoglobins (Koch et al., 1964), liver proteins (Hopper and Yatvin, 1965; Narayansingh and Eales, 1975) and plasma proteins (Enomoto, 1964; Takashima et al.,1972). More direct evidence comes from increased ^{14}C aminoacid incorporation into protein as a result of T_3 or T_4 treatment (Thornburn and Matty, 1963; Jackim and La Roche, 1973; Narayansingh and Eales, 1975). Narayansingh and Eales have followed ^{14}C-leucine incorporation into gill, liver and plasma proteins of trout and conclude

that that both T_3 and T_4 increase protein synthesis and the liver protein content.

It is further evident from the results (Section 4.2.2) that the effects of thyroid hormones are pituitary dependent because both T_4 and thiourea were effective in causing significant alterations in ova diameter measurements, ovarian protein, GSI and liver protein values in intact but not in hypophysectomised regressed fish. Similar pituitary dependent T_4 action in ovarian maturation has also been observed in goldfish (Hurlburt, 1977).

Although effects of thyroid on metabolism appears to be one of the best established function of thyroid gland there is evidence for some involvement of thyroid hormones in carbohydrate metabolism too. T_4 administration reduced the glycogen contents in liver (Fontaine et al.,1953; Le Ray et al.,1970; Bhatta and Khanna, 1976) and increased in heart and muscle (Murat and Sarfaty, 1971; Bhatta and Khanna, 1976). T_3 and ipodate were ineffective in causing significant alterations in HSI values of S. gairdneri (Cyr and Eales, 1988a). In this study T_4 depressed liver glycogen and HSI values while thiourea treatment increased both liver glycogen and HSI contents in intact H. fossilis. The effects of both T_4 and thiourea are again pituitary dependent as pituitary ablation vanishes this response. Ball and Hawkins (1976) have observed similar findings in Poecilia after immersion them in T_4. Thyroxine induced low liver glycogen contents as reported in the present investigation may be due to its decreased synthesis and/or rapid degradation by T_4.

Both temperature and/or season can modify response in carbohydrate metabolism to thyroid hormones (Fontaine et al., 1953; Murat and Surfaty, 1971). The present study was carried out in late post-spawning and preparatory phases. It is observed that SG-G100 treatment caused significant increase in liver protein while liver glycogen was reduced. These effects of SG-G100 were augumented when SG-G100 was used in combination with T_4. These findings are similar to those observed in untreated intact fish during spawning phase (Lal and Srivastava, 1989). It seems that SG-G100 induces physiological conditions in these experimental fishes similar to that of spawning phase and therefore, the above responses of SG-G100 towards the protein and glycogen, as observed here, is a manifestation of the physiological effects of the hormone.

It is remarkable to observe that the ovarian glycogen contents in intact as well as hypophysectomised fishes did not alter under any experimental conditions. However, during the seasonal cycle studies, ovarian glycogen were reported to show variation during the different seasons and maximum glycogen values were found during June-July (Lal and Srivastava, 1989) when temperature and photoperiod were maximum. The present findings that the ovarian glycogen remained unchanged under all the experimental manipulations as specified in the text might be a seasonal effect of thyroid hormone on carbohydrate metabolism as the present experiment was carried out during November to February when temperature and photoperiod were minimum.

Since thiourea is also known to block thyroid hormone synthesis, its reverse effect on liver glycogen when compared to T_4 as reported here is in agreement according to its mode of action. Thus thiourea led increase in liver glycogen may be due to its less utilization. It is further apparent that the influence of both thyroxine and thiourea on liver glycogen synthesis is reflected well in HSI values.

HSI values have been shown to increase after hypophysectomy in normal and untreated fish as a result of increased liver glycogen stores (Walker and Johensen, 1975; Ball and Howkins, 1976; Hurlburt, 1977). In this investigation also similar observations have been recorded. Since during gonadal maturation, increase in ovarian weight is several times higher, the energy and nutrients requirements must be very high. It is likely that during this time liver glycogen is the possible source for high energy and nutrients demand. Petersen and Emmersen (1977) have observed that liver glycogen and lipid represent the energy source to meet the metabolic needs during growth and spawning. Verma et al. (1985) opined that liver glycogen may take part in yolk formation. Therefore, it appears that the effect of thyroid hormone on carbohydrate metabolism during ovarian maturation is well exercised.

Hypophysectomy has been shown to arrest vitellogenesis in many teleosts (Barr, 1963; Yamazaki, 1961, 1965; Sundararaj and Goswami, 1968). It has been suggested that vitellogenesis is under control of gonadotropin stimulation through ovarian

estrogen. Therefore, pituitary ablation would account for removal of gonadotropin leading to inhibition of steroidogenesis. In H. fossilis also hypophysectomy resulted in lower GSI, ovarian protein and ova diameter. This might be due to diminished incorporation of yolk into oocytes as suggested by Campbell and Idler (1976).

It has been suggested that the involvement of thyroid in reproduction may be due to its synergistic action with the gonadotropin rather than T_4 directly stimulating pituitary function. In stellate sturgeons after prolonged captivity and cooling, T_3 was effective in restoring ovarian response to hypophyseal stimulation (Detlaf and Davydova, 1974). Hurlburt (1977) observed that ovarian response to SG-G100 increased more in hypophysectomised with T_4 than alone. Epler and Beiniarz (1983) recorded that T_3 + steroid hormone or T_3 + gonadotropic hormone increase percentage of mature oocytes than steroid hormone or gonadotropin treatment alone in carp, Cyprinus carpio and suggested that T_3 makes oocytes more sensitive to the effects of steroids or gonadotropic hormones. In rainbow trout, Cyr and Eales (1988a) observed that increase in plasma T_3 concentration at physiological dose level increased ovarian response to estradiol release. They have further recorded that increase in GSI in response to gonadotropin treatments is more when gonadotropin is given in combination with T_3 than gonadotropin alone.

A synergistic action of thyroid hormones and gonadotropins on reproduction is recognized in mammals. In this group, thyroid hormone may stimulate gonadal development in intact individuals and augment the ovarian response to exogenous gonadotropin stimulation both in vivo and in vitro (Leatham, 1972; Channing et al.,1976); alone, however, thyroid hormones are ineffective in influencing gonadal maturation after pituitary ablation (Eartly and Leblond, 1954).

In the present study, it is evident that T_4 was capable of increasing the ovarian response to SG-G100 towards ova diameter, ovarian protein synthesis and GSI after hypophysectomy. This lends further support to the above suggestion of synergistic mode of action of thyroid hormone. However, considering both direct and indirect actions of T_4 on the ovary, it is likely that T_4 might serve to metabolise necessary precursors for ovarian maturation such as availability of nutrients, metabolites and yolk or effect of ovarian metabolism directly probably by way of increasing ovarian sensitivity to gonadotropin stimulation. In the present investigation SG-G100 given either alone or incombination with T_4 did not change the thyroid activity hence it may be safely concluded that SG-G100 was not contaminated with TSH.

5.5 Influence of sex steroids on thyroid gland, ovary and liver of H. fossilis

Influence of thyroid hormone on reproduction of teleosts appears to be well established. The reciprocal relationship between

thyroid and reproduction is due to the effects of gonadal hormones on the thyroid rather than thyroxine affecting reproduction. Effects of sex steroids on thyroid gland in fish has been reported by several workers. Matty (1960) observed that in S. squalidum complete hypertrophy of the thyroid gland occurred as evidenced by several times higher epithelial cell height following testosterone phenyl acetate or methyl testosterone treatments. Singh (1968,1969) recorded increased radioiodine uptake in response to different gonadal and cortical steroids in both intact and hypophysectomised M. vittatus. Nishikawa (1976) noticed signs of higher histological activity in thyroid gland of O. latipus after methyl testosterone treatment. Bandyopadhyay and Bhattacharya (1988) and Bandyopadhyay et al. (1991) treated C. gachua with estrogen and found T_4 release. Thus our present findings that different sex steroids enhanced thyroid activity index in H. fossilis is in concurrence with the above reports.

However, it is apparant from the results that methyl testosterone significantly enhanced the TAI values at 2.50 µg and 5.0 µg/g body weight dose levels while testosterone propionate was effective at 5.0 µg/g body weight dose only. It is likely that methyl testosterone is more potent than testosterone propionate in inducing such changes. Several workers have also reported higher androgenic potency of methyl testosterone in teleosts (reviewed by Bales, 1979). Annual reproductive cycle also revealed that during prespawning and spawning phases of H. fossilis large quantities of the gonadal steroids are released

in the blood (Singh and Singh, 1990) and at that time thyroid activity was maximum (Lal and Srivastava, 1990a). Therefore, it seems that released gonadal hormones influence the thyroid activities during reproductive phases. Our observations of enhanced thyroid activity index following different sex steroids treatments in H. fossilis lends further support in favour of the supposition of reciprocal relationship between thyroid and gonad atleast in this species.

Thyroid activity was found to be unaltered in gonadectomised C. batrachus (Lehri, 1966). However, our results of suppressed thyroid activity in ovariectomised H. fossilis is in agreement with those of Overbeeke and McBride (1971), who have also recorded histological signs of depressed thyroid activity in O. nerka following gonadectomy.

Matty (1960) suggested that androgens act directly upon thyroid gland and not as a result of thyrotropin stimulation. Singh (1968, 1978) reported that in M. vittatus gonadal hormones increased thyroid activity and this effect was not mediated through the pituitary gland. Sage and Bromage (1970) working on P. reticulata also suggested that influence of androgens upon thyroid gland is probably a direct one and does not involve the pituitary gland. In the present investigation also no sign of histological stimulation or activity in pituitary thyrotrops were noticed in H. fossilis. This lends further support to the above findings. It may be therefore, concluded that in H. fossilis sex steroids influence thyroid activity in a direct manner without effecting thyrotrops.

In most of the nonmammalian vertebrates, yolk proteins and precursors are of extragonadal origin. Although hepatic synthesis of yolk protein is influenced by estrogens (Hori et al., 1979; Scott et al.,1980; Ng and Idler, 1983; Mitra and Nath,1988) which are secreted into the blood and cause elevation of plasma protein level (Aida et al.,1973; Emmersen and Emmersen, 1976; Olivereau and Olivereau, 1979; Campbell and Idler, 1980). The serum concentration of androgens like testosterone and keto-testosterone and estrogens like estradiol and progesterone were extremely high throughout the course of vitellogenesis, gonadal development and spawning migration in the fish and at that time plasma protein levels are increased (Yaron et al., 1977; Kagawa et al., 1983; de Vlaming et al.,1984; Feist et al.,1990). In the present investigation hepatic protein contents were increased following the treatments with MT, TP, E and P. It may be either due to increased vitellogenesis or less degradation of protein. However, androgen like testosterone may be serving as precursor for estrogen synthesis (Callard et al.,1978, 1981; Kagawa et al., 1982; Nagahama et al.,1991). Therefore, it is likely that MT and TP acted via estrogen as reported by LeMenn et al. (1980). A similar results concerning in increased liver protein have been obtained in flounder (Emmersen and Petersen, 1976) and cod (Black et al.,1971) when treated with estrogen. Estrogen induced hypertrophy of liver cells in flounder, P. flesus (Emmersen and Petersen, 1976), winter flounder, P. ammericanas (Campbell and Idler, 1980) and catfish, H. fossilis (Medda et al., 1980; Nath and Sundararaj, 1981). This hepatic hypertrophy

might have been reflected in increased level of protein contents of liver. Medda et al. (1980) have reported that testosterone failed to cause any significant changes in protein content of liver whereas estradiol increased this value. They further opined that the difference between the actions of testosterone and estrogen on hepatic protein levels might have been due to the differences in hormonal dose and duration of the experiment.

Both methyl testosterone and testosterone propionate increased the ovarian protein contents while estradiol and progesterone failed to cause any significant variations in the protein content of the ovary. Increased amount of protein in ovary may be due to the deposition of extragonadal or intragonadal originated proteins following the treatment with MT and TP. Our observations indicate that male sex hormones behave differently than the female sex steroids with respect to ovarian protein contents. It is assumed that androgens like MT and TP act as estrogen precursors in fish ovary and further not all the TP is converted into the estrogen, but the part which remains unutilised is secreted in the blood plasma as TP (reviewed by Fostier et al., 1983). This unutilised TP might be involved in the ovarian protein synthesis leading to its accumulation. Our observations of differential action of androgen on ovarian protein content with respect to estrogen seem to favour such function of TP in ovarian protein incorporation.

Earlier reports have indicated that estrogen and its derivatives have no role in stimulatory terminal maturation of primary oocytes either in vivo or in vitro (Goswami and Sundararaj, 1974; Sundararaj and Goswami, 1977). Medda et al. (1980) assumed that estrogen may be responsible for causing early maturation of primary oocytes by inducing formation and secretion of yolk protein precursor from liver. In our observation estrogen and progesterone though did not alter ovarian protein content but they did increase hepatic protein levels. This lends further support to the above mentioned view of Medda et al., 1980. Incorporation of yolk protein into oocytes is gonadotropin dependent in oviparous vertebrates (Idler and Ng, 1979; Burzawa-Gerard, 1981; Sundararaj et al.,1982). Many workers favour the concept of negative feedback at the level of hypothalamus exerted by estrogen on pituitary gonadotropin (Egani, 1955; Egani and Ishii, 1962; Sundararaj and Goswami, 1968). Therefore, inhibitory role of estrogen and gonadotropin secretion might have resulted in the unaltered level of ovarian protein and GSI in H. fossilis as reported here. Some report also demonstrated that estrogen has no effect in inducing GSI (Black et al., 1971; Campbell and Idler, 1976; Medda et al.,1980) whereas reduction in GSI in H. fossilis was obtained in response to estrogens (Sundararaj and Goswami, 1968).

In the present study both MT and TP increased the hepatic as well as ovarian glycogen content suggesting an anabolic role of these hormones in H. fossilis. Similarly, Dasmahapatra and

Medda (1982), Lal and Srivastava (1990b) have reported anabolic effect of testosterone in liver and Power and Florini (1975) in muscle of fishes. The role of male steroids on carbohydrate metabolism has also been demonstrated (Guerrereo, 1975; Yamazaki, 1976). During vitellogenesis, gonadal growth and spawning, high energy is required for muscular contraction (Love, 1970; Mackinnon, 1972; Petersen and Emmersen, 1977; Lal and Srivastava, 1989), this necessiates the storage of glycogen in the liver and gonad. It is evident that MT induced changes in protein and glycogen contents of liver and ovary at different dose levels tested were of higher magnitude than the TP. Hence, it is likely that MT is more potent than TP in inducing such changes. Both female sex steroids reduced the liver glycogen content indicating the catabolic role of these hormones in this species. The observed decrease in hepatic glycogen following female sex steroids treatment might have been due to its enhanced mobilization and/or less synthesis. Similar results have been reported in brook trout (Whiting and Wiggs, 1978), goldfish (Ishii and Yamamoto, 1970) and Singi fish (Basmahapatra and Medda, 1982). Sand et al. (1980) found no change in hepatic glycogen contents following estrogen treatment. Basmahapatra and Medda (1982) recorded increase in hepatic lipid contents after estrogen injection in H. fossilis. Since liver glycogen is utilised during lipogenesis, the observed decrease in liver glycogen content following E and T treatment in the present study might be associated for the utilization of liver glycogen towards the lipogenesis.