DISCUSSION
DISCUSSION

Although in developed countries the tendency is to reduce the excessive productivity of water bodies, in developing countries enhancement of productivity to increase the food source is the main aim. Inland fisheries meet the requirement for both in the developed countries as an instrument for biomanipulation, and in the developing countries as a source of food.

Like most agricultural production, aquaculture has to grow to meet the increasing market opportunities for the producers and the distributors of aquaculture products. Fish is the source of animal protein in many countries and for many millions, particularly in poorer sections of the community. Increased interest in fish both for food and for ornamental purpose has prompted an awareness of problems associated with their health. A recent survey has indicated that consumption of fish has increased for health reasons (Gutting 1990), and also for the presence of less connective tissue than other animal flesh. Fox (1978) and Browne (1990) have reported cholesterol lowering properties and decreasing blood clotting activity through fish food

Though toxicology tests are critical, economic, social and scientific importance have not been acknowledged by toxicologists, regulators and the public. Professionals have historically placed emphasis on longterm repeat exposure studies which have seen as providing definitive answers to health effects associated with extreme dosages or exposure in acute studies.
The aquatic toxicology test considers fish as a representative of the biota, and is considered as a screening test. It determines the presence or absence of harmful wastes, which is ascertained by the survival of fish in the sample water.

The significance of the interaction of toxicological effects in animals is well discernible from the studies of Murphy and Dubois (1957), Frawley (1965) and Cohen and Murphy (1974).

Though it is found to be useful in the control of insects and pests, its effect on non-target organisms of aquatic habitat are more hazardous. For instance, if one chemical is applied for control of larval stages in insect or pests, and another to control aquatic algae or rooted vegetation, some knowledge on the toxicity of such pesticide combination is necessary to protect fishery resources and to establish safe dosage patterns for these chemicals (Macek, 1975).

The routes of pesticide transport to different aquatic ecosystems has been well documented by Nicholson (1970). It is well known that various pollutants, including pesticides, will be carried to the freshwater by means of different processes like surface run-off, disposal through wastes, spray drift and direct application. All these methods of translocation of pesticides may occur at a time or they may occur individually at different times.

Effluents have posed a serious threat to the vast and varied fishery resources of the country. Water quality of major rivers is getting rapidly degraded due to discharge of industrial wastes of diverse origin, domestic sewage, flyash, mine drainage, oil seepages, dumping of radio active materials in sea. Extensive use of insecticides and pesticides on the practice of agriculture too have polluted the water.
Pollution of aquatic environment by domestic wastes and untreated or partially treated industrial effluents, both acidic and alkaline supplemented with other pollutants like heavy metals, pesticides and many other organic compounds greatly contribute to the massive fish kills and other important members of aquatic biota. Recently there have been several reports on fish mortality which has been attributed largely due to pollution affecting various organ system acutely or chronically (Manjuladevi 1988).

Since gills are the primary route of entry for pesticides and also sensitive to infectious as well as non-infectious agents, it is suggested that a study of morphology, histology and histopathology of gills could be a good indicator of health condition of fish population.

It is well known that thyroid hormones play an important role in the various metabolic activities of animal. Further it is believed that the thyroid hormone together with other hormones are involved in osmoregulation and gonadal maturation in fishes. For instance, in teleosts annual cycle of thyroid activity has been correlated with gonadal cycles. (Berg et al. 1959; Ichikawa et al. 1974 and White and Henderson 1977). As reported by Sage (1973), these cycles as well as altering sensitivity to the environment may serve to regulate gonadal development. Other well documented examples by White and Henderson (1977) have evidenced an increase in thyroid activity during the phase of reproduction and spawning. However, there has been difficulty in providing a direct thyroid–gonadal interrelationship in fishes.
It may be hypothesized that traditional and nutritional patterns have led to the evolution of aquaculture on various parts of the world. As the freshwater fishes in India constitute an important source of protein in rural as well as urban areas, an understanding about the deleterious effects of pesticides in fishes and their safe permissible concentration in the aquatic environment would be more rewarding for fish conservation and development of aquaculture. In aquatic environment the pesticides get mixed up to sublethal levels, producing chronic histopathological effects on different organisms. They are readily absorbed through gills which bring metabolic imbalances in different systems. It is in this context that a study of gills will be more relevant to evaluate the degree of damage caused by a commonly used pesticide like malathion.

Histopathological observations were made in the gills of *G. giuris*, because of its sensitive nature, and its respiratory surface coming into the direct contact with the pesticides.

The result of the present investigation on gills of *G. giuris*, after exposing them to lower concentrations of malathion during non-breeding phase showed morphological changes in the gill filament, separation of respiratory epithelium, degeneration of primary and secondary lamellae along with vacuolization. Similar observations were made by Patil (1987) in *Tilapia mossambica* exposed to malathion, Pandey et al. (1996) in *Liza parsia* after sublethal exposure to mercuric chloride, Vijayalakshmi and Tilak (1996) in *Labeo rohita* after treatment with pesticides and Dutta (1995) in *H. fossilis* after treatment with diazinon.

The results obtained from higher concentrations of malathion indicated
lamellar talangectases, hyperplasia of chloride cells, fusion and shortening of secondary lamellae. The above findings coincide with that of Pandey et al. (1997) in *Liza parsia* after treatment with BHC and Randi et al. (1996) in freshwater fish *Macropsobrycon uruguayanae* after exposing them to cadmium.

Exposure of fishes to sublethal concentrations during breeding period elicited excess mucus secretion, histopathological changes like fusion of secondary lamellae, vacuolization and degeneration of primary lamellae in the gobiid, *G. giuris*. Similar observations have been made by Phromkunthong (1993) in *Epinephelus malabaricus* after exposing them to ascorbic acid, and Manoj (1999) in *Boleophthalmus dussumieri* after exposure to cadmium. The hyperplasia, necrosis, desquamation of the epithelial cells and the talangectases of gills are observed by Randi et al. (1966) in *Macropsobrycon uruguayanae* after prolonged treatment with cadmium. Such a phenomenon has been noticed in *Salmo salar* (Marius et al. 1996) due to the presence of hydrogen peroxide and in *Liza parsia* after mercuric chloride intoxication (Pandey et al. 1996).

Kumar and Pant (1984) remarked that histopathological studies were useful in evaluating the pollution potential of pesticides. They opine that even though trace levels of these chemicals which did not bring about animals mortality over a given period, are quite capable of producing considerable organal damage.

Severe oedema, increased interlamellar space and fusion of secondary lamellae after prolonged treatment (96 hrs) of malathion occurred in *G. giuris*. Similar observations on gill have been noticed by Manoj and Ragothaman (1999) in the estuarine fish, *Boleophthalmus dussumieri* (Cuv.) after exposing them to sublethal
levels of cadmium; Kiemer and Black (1997) in *Salmo salar* (L) after exposing them to hydrogen peroxide; Kumaraguru *et al.* (1992) in rainbow trout *Salmo gairdneri* after treatment with permethrin; and Pandey *et al.* (1997) in *Liza parsia* by sublethal exposure to BHC.

In the present investigation, toxic effects of different concentrations of the organophosphorous pesticide malathion were made in the thyroid gland of *G. giuris* after exposing them for a period of 24-96 hrs. Treatment with sublethal levels of malathion (0.05 and 0.5 ppm) for 24 hrs in *G. giuris* elicited several changes in the thyroid including cellular hypertrophy, follicular hyperplasia, and changes in epithelial cells with dense colloid. Many vacuoles were found in the colloid with the decrease in follicular diameter, epithelial cell height and height of colloid. Similar responses to various pesticide pollutants have been observed by Wani (1984) in cyprinid fish, *Garra mullya* (Sykes); Rao and Mukherjii (1972) in *Heteropneustes fossilis*: Srivastava and Sathyanesan (1971) in *Mystus vittatus* after thiourea administration. Leatherland and Sonstegard (1980) in *Onchorynchus kisutch*, Singh *et al.* (1974) in the airbreathing fish *Heteropneustes fossilis* while studying various phases of seasonal cycles, and van Overbeeke and Mc Bride (1971) in gonoatectomised sockeye salmon *Onchorhynchus nerka*. After treatment with 11-ketotestosterone, 17 alpha-methyl testosterone, estradiol, estradiol cypionate and cortisol. After treatment with 0.05 and 0.5 ppm of malathion for 96 hrs the follicular lumen was obliterated due to hyperplasia and hypertrophy of epithelial cells. In some follicles the follicular lumen was totally obliterated with the loss of colloid, while in others they were found with little amount of colloid. Similar observations
were made by van Overbeeke and Mc Bride (1981) in gonadectomised sockeye salmon *Onchorhynchus nerka*, and by Baker-cohen (1961) in platyfish. In the present study on *G. giuris* it was observed that malathion treatment produces a hyperthyroid conditions. This finding coincides with that of Ragneker and Latey (1937) in *Tilapia mossambica*. Further, this malathion had caused damages on the structure of the follicle, amount of colloid, epithelial cell height and function of the cell.

Pesticides become toxic to non-target organisms in the aquatic system and may cause several imbalances in the ecosystem as well. In the present investigation, the toxic effect of different concentrations of organophosphorous pesticide malathion were made on the thyroid gland of *G. giuris* after exposing them for a period of 24-96 hrs. The follicular size of the thyroid and epithelial cell height got reduced after malathion treatment. The hyperactivity of thyroid follicles during breeding period appears to correlate with gonadal maturation (White and Henderson, 1977). Not much attention has been given to the activity of thyroid hormone after pesticide toxicity. In *G. giuris* presence of colloid in the lumen of thyroid follicles in the breeding phase was higher when compared to those in non-breeding period after malathion treatment.

The fish *G. giuris* was exposed to various percentages of thiourea (0.01, 0.03 and 0.05 %) for a period of 7, 14 and 21 days. It is clear that thiourea treatment, induced an increase in the number and size of the follicles. These changes were accompanied by increase in the blood supply to the thyroid tissue. These observations together with hyperplasia, hypertrophy, and hyperemia of the thyroid

After treatment with thiourea for 7 days the thyroid follicles exhibited peripheral vacuoles and a progressive increase with the increased percentage of thiourea. When fishes were exposed for 14 days, thyroid exhibited hypertrophied epithelium, follicular atrophy, oedema and hypertrophy of thyroid follicles. Similar results were observed by Wani (1984) in Garra mullya. After 21 days of exposure to thiourea it was evident that the toxic effect could be due to prolonged duration with an increase percentage. The results include marked diminution of follicular size and lumen of the follicles with concomitant loss of colloid with few peripheral vacuoles. Such anti-gonadal effects of thiourea have been recorded by Pandey and Leatherland (1970) in Poecilia reticulata and Mukherjee (1975) in Heteropneustes fossilis.

The thyroid of G. giuris revealed that it is active during breeding phase. This finding suggests that in G. giuris thyroid gland may exert a positive effect on the reproductive function of the fish. Similar results have been reported by Srivastava and Sathyanesan (1971) in Mystus vittatus and Rangnekar and Latey (1977) in Tilapia mossambica.

The gill is the most important organ for respiration and osmoregulation. It is the first organ to which the pollutants come into contact and hence it is more vulnerable to damage than any other tissue. In higher concentrations (0.05 %) of thiourea gills exhibited hyperplasia of primary and secondary lamellae. Similar results were reported by Chauhan Pandey (1987) in the gills of Puntius ticto; Baticodius (1991) in Penaeus monodon after gusthion treatment and Drewett (1983)
in *Salmo trutta* after lindane poisoning; Paperna and Vanvas (1983) in *Chilodonella hexasticha* and Marius *et al.* (1997) in *Salmo salar* after treatment with hydrogen peroxide. Sunitha and Sahai (1993) reported inflammation in respiratory lamella in the gills of *Rasbora daniconius* after exposed to Gama-BHC. Hyperplasia and fusion of gill filaments due to separation of epithelium, degeneration of pilaster cell and development of vacuoles in the epithelium are pathological changes observed in thiourea exposed fish. Similar changes have been found to occur in *Tilapia mossambica* (Radhaiah 1988) after exposed to fenvalerate, Kumaraguru *et al.* (1982) in *Salmo gairdneri* with Permethrin and Nath *et al.* (1989) in *Colisa fasciatus* after exposed to nickel. It has been pointed out by Alan (1974) that such secretion might protect the fish against infection (mucus secretion after exposure to thiourea has been observed in *G. giurus*).

Histochemical investigations with respect to proteins and carbohydrates in the healthy as well as in the treated fishes have been carried out, since very few works have been done in the past viz., Nazmi 1986; and Jebakumar *et al.*, 1996. Further, only little work has been done with regard to localisation of the chemical substances in the different parts of thyroid. Hence in the present study localisation of proteins and carbohydrates were carried out in the thyroid gland of control, malathion treated and thiourated fishes.

In the present investigation bromophenol blue (Hg-BPB) test was employed in thyroid of *G. giurus* for localising proteins in control and malathion treated fishes. The thyroid of control fishes showed moderate reaction in colloid content indicating the presence of lesser proteins. However, in the thyroid of malathion intoxicated
fishes, during non-breeding period, an increase in protein content was observed after treatment with 0.5 ppm malathion for 24 hrs. But protein contents were found to decrease after prolonged malathion treatment (for 96 hrs) in the colloid content, while in the epithelial cells it had got reduced. (No further variations were noticed after 48 and 72 hrs of exposure). Similar observations were made by Lata (1995) in the thyroid of Oreochromis mossambicus after treatment with endosulphan.

In breeding phase reduction in protein content was observed in the epithelial cells and in the colloid of thyroid after 96 hrs exposure to 0.5 ppm malathion. Similar observations were made by Bara (1972) in Pseudopleuronectes americanus while studying histochemistry of enzyme dehydrogenase in the testis.

In thiourated fishes upto 21 days (during non-breeding phase) protein content showed a decreasing trend in the colloid of thyroid gland, while lesser to moderate contents were noticed in the epithelial cells.

In 0.05 ppm to 0.5 ppm malathion intoxication, during non-breeding phase upto 72 hrs duration the results were similar to controls. After 96 hrs negative reactions for carbohydrate in the colloid contents of the thyroid gland was noticed. In the epithelial cells a slight decrease of carbohydrate was noticed after 0.05 ppm malathion intoxication for 48 and 96 hrs. In the present findings carbohydrate deposition in the thyroid gland indicated decreasing pattern with an increase in the concentration of pesticides. Similar observations were noticed in the thyroid gland of breeding phase. While G.giuris were treated with 0.01 to 0.05% thiourea for 7 to 21 days duration decreasing trend of carbohydrate was noticed in the thyroid with an increase in the percentage of thiourea. Further reduction of carbohydrates was
noticed in epithelial cells as evidenced by positive to moderate reaction for PAS. Similar reactivity of this test was reported by Pandey (1975) while studying the histochemical nature of ectopic thyroid follicles in *Osteobrama cotio*.

During breeding phase (with 0.01% thiourea) only colloid content of 7 and 14 days thiourated fishes showed positive reaction, while after 21 days of exposure, PAS test for carbohydrate contents showed a negative reaction. Present studies revealed heavy reduction of carbohydrate contents in 21 days of treatment with 0.01 and 0.05% of thiourea. Similar observations have been made by Misra (1990) in *Channa gachua* during non-breeding and breeding phase.

In the present investigation, Hg-BPB test was employed in the gills of *G. giuris* for locating general proteins in control and malathion treated fishes. The gills of control fishes showed positive reaction for Hg-BPB in mucous and chloride cells indicating a larger deposition of proteins in these cells, while the pillar cells showed moderate reaction.

In the gills of malathion treated fishes, during non-breeding phase, reduction in protein content was observed after 0.5 treatment for 24 hrs in chloride cells, which had increased after 96 hrs of treatment. In the pillar cells protein content were slightly reduced after 72 hrs of exposure with 0.5ppm malathion. Mucous cells of gills showed similar results. Such a phenomenon has been observed by Harris *et al.* (1973) while studying histochemical anlaysis of the epidermis of brown trout *Salmo trutta*.

In the secondary lamellae of gills, during breeding period, reduction in protein content was noticed after 24, 48 and 96 hrs of treatment for with 0.05 to 0.5 ppm of
malathion. Interestingly in the pillar cells protein content had increased after 24 and 72 hrs exposure.

In the present findings during non-breeding phase, slight increase of carbohydrates was seen in the mucous cells of secondary lamellae after 24, 48, 72 hrs of treatment for 0.05 to 0.5 ppm malathion. The chloride and pillar cells on the other hand indicated reduction in carbohydrate content during 24 hrs exposure to 0.05 to 0.5 ppm malathion. Similar observations have been made by Alan et al. (1977) in char, *Salvelinus alpinus* while studying histochemistry of mucous cells.

A decrease of carbohydrate content was observed in the mucous cells after 24, 48 and 96 hrs for 0.05 to 0.5 ppm malathion treatment, while in chloride cells an increase of same was noticed in experiments conducted for 48 and 96 hrs of duration. Pillar cells of gills showed strong positive reaction during breeding phase. Similar observations were made by Kumari and Kumar (1997) in *Channa Punctatus* while studying the effect of polluted water on histochemical localisation of carbohydrates.

In the secondary lamellae of control fish during non-breeding phase, mucus cells gave negative reaction for Hg-BPB test during 7,14 and 21 days of exposure to thiourea, (0.01% to 0.05%) while chloride cells showed a positive reaction indicating an increase of proteins. The results obtained from pillar cells were similar to that of chloride cells (except for 14 days treatment).

In the gills protein contents were found to be increased in pillar, chloride and mucous cells of control fishes. Depletion of proteins was recorded in mucous cells, as they were negative in reaction. No further variations was observed in pillar cells after 7-21 days of exposure to 0.01, 0.03 and 0.05% of thiourea treatment.
The secondary lamellae of control gills of *G. giuris* during non-breeding phase indicated accumulation of carbohydrates in pillar, chloride and mucous cells. In the mucous cells, carbohydrate contents were increased after 7-14 days of exposure to 0.01 to 0.05 ppm of thiourea treatment. Whereas in chloride cells depletion of carbohydrates was recorded for 14-21 days of 0.01 to 0.05% of thiourea treatment. No further reactivity was observed in pillar cells. Similar observations were noticed by Dezwann *et al.* (1972) in *Mytilus edulis*, Alan and Pickering (1974) in char *Salvelinus alpinus*.

Histochemical investigations with respect to carbohydrates in the secondary lamellae of gills of gobiid fish *G. giuris* have been carried out during breeding phase. The pillar and chloride cells indicated a higher deposition of carbohydrates in them. In mucous cells lesser deposition of carbohydrates was observed. However in mucous cells the carbohydrate contents increased after 7-21 days of 0.01, 0.03 and 0.05% of thiourea treatment. While the chloride and pillar cells exhibited depletion of carbohydrate contents in 0.01 and 0.03% of thiourea treatment for 14 and 21 days. Similar observations were made by Harris, *et al.* (1973) in brown trout *Salmo trutta* and Lehtonen *et al.* (1965) in *Myxine glutinosa*. 