2. Review of Literature

2.1. General Review

Pesticide toxicity is one of the major environmental problems of the present day world. Although pesticides namely organochlorines, organophosphates, carbamates and synthetic pyrethroids are used widely in agriculture for controlling pests, their residues often reach aquatic ecosystems through agricultural runoff (Das and Mukherjee, 2003). Synthetic pyrethroids are synthesized derivatives of naturally occurring pyrethrins that are obtained from the flower of a plant, Chrysanthemum cinerariaefolium (Güneş and Yerli, 2011; Ansari and Ansari, 2012). They are among the major insecticides used in agriculture and household pest control (Mujeeb and Shakoori, 2012). Although synthetic pyrethroids are preferred to organochlorines, organophosphates and carbamates due to their low toxicity to birds and mammals, high potency and effectiveness, easy biodegradability, and low persistence in the environment, they are reported to be highly toxic to non-target aquatic organisms including fish (Bâşer et al., 2003; Ural and Sağlam, 2005; Singh et al., 2010; Cao et al., 2011). Thus, synthetic pyrethroids are one of the major contributors of aquatic pollution which are reported to be extremely toxic to fish (Richterová and Svobodová, 2012). Moreover, since fish serves as a major food source of humans, any effect of pesticides in fish ultimately affects humans through the food chain. Therefore, a review of the effects of synthetic pyrethroid insecticides on several toxicological end-points in fish is provided here.

2.2. Acute Toxicity of Pyrethroids including Deltamethrin and Permethrin on Fish
Acute toxicity of permethrin to brook trout and rainbow trout revealed that permethrin was toxic to both the fish species where the 96 h LC$_{50}$ values were estimated as 3.2 µg l$^{-1}$ and 0.6 µg l$^{-1}$, respectively (Johnson and Finely, 1980; Kumaraguru and Beamish, 1981). Tilak et al. (1981) studied the toxicity of carbaryl and 1-naphthol to four species of freshwater fish, viz., *Catla catla* (Ham.), *Anabas testudineus* (Bloch), *Mystus cavasius* (Ham.) and *Mystus vittatus* (Bloch) and noted that the carp, *Catla catla* which was supposed to be a very sensitive fish, incapable of withstanding environmental stress, was the least sensitive of the four fish species to both carbaryl and 1-naphthol. Holcombe et al. (1982) also investigated the acute toxicity of permethrin for rainbow trout (*Salmo gairdneri*) and fathead minnows (*Pimephales promelas*) where the 96 h LC$_{50}$ values were estimated as 7.0 and 15.6 µg l$^{-1}$ respectively. Besides, Haya (1989) carried out investigation on the biological, chemical and environmental factors that affected the acute and chronic, lethal and sublethal toxicity of pyrethroid insecticides to fish and reported that pyrethroids were extremely toxic to fish where the 96 h LC$_{50}$ values were in the nmol l$^{-1}$ range. He further added that the effects on nervous system, respiratory surfaces and renal ion regulation were associated with the mechanism of lethal action in fish. Görge and Nagel (1990) investigated the toxicity of deltamethrin, lindane and atrazine to early life stages of zebrafish (*Brachydanio rerio*) and reported that the early life stages were less sensitive to lindane, whereas in the case of atrazine and deltamethrin, larvae were more sensitive. Kumar et al. (1999) determined the 96 h LC$_{50}$ value of deltamethrin for *Heteropneustes fossilis* as 0.52 mg l$^{-1}$ and further reported induced physiological changes in the fish. Rahman et al. (2002) investigated the effect of diazinon 60 E.C. on *Anabas testudineus, Channa punctatus* and *Barbodes gonionotus* where their LC$_{50}$ values indicated that diazinon had more effect on *Barbodes gonionotus* followed by *Channa punctatus* with *Anabas testudineus* being the most tolerant. Bayer et al. (2003)
investigated the acute toxicity of permethrin on guppy fish (*Poecilia reticulata*) where the 48 h LC$_{50}$ value was estimated as 245.7 µg l$^{-1}$ using probit analysis. They also reported that the acute toxicity value of permethrin for various other fish species was in the range of 0.05-97.0 µg l$^{-1}$.

Further, Svobodová et al. (2003) studied the acute toxicity of deltamethrin on fish (*Cyprinus carpio* L.) and stated that based on 96 h LC$_{50}$, deltamethrin could be included in the group of substances that are highly toxic to fish. Viran et al. (2003) investigated the acute toxicity of deltamethrin on guppy fish (*Poecilia reticulata*) where the 48 h LC$_{50}$ value was estimated as 5.13 µg l$^{-1}$ using probit analysis. Deltamethrin exposure to *Cyprinus carpio* L. embryos and larvae resulted in mortality at lethal dose, while sublethal dose caused decrease in hatching success of fish indicating that the pesticide had an adverse effect on the reproduction and development of this carp. These facts, therefore, needed to be considered when this chemical was used in agricultural areas near aquatic ecosystems (Köprüçü and Aydın, 2004). Ural and Sağlam (2005) found the 96 h LC$_{50}$ value of deltamethrin to the fry rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) to be 0.6961 µg l$^{-1}$ and concluded that deltamethrin is among the highly toxic pesticides for fish where the adverse effects depend on concentration and duration of exposure.

Boateng et al. (2006) also carried out acute toxicity of deltamethrin on fish, *Oreochromis niloticus* (Linnaeus, 1758) where the LC$_{50}$ values was 15.47 µg l$^{-1}$ and reported that deltamethrin was highly toxic to the fish which adversely affected its physiology. Köprüçü et al. (2006) found the 96 h LC$_{50}$ value of deltamethrin to the fingerlings of European Catfish, *Silurus glanis* L. to be 0.686 µg l$^{-1}$, which further proved the highly toxic nature of deltamethrin. Besides the aforementioned studies, acute toxicity studies on toxicants other than pyrethroids on *Anabas testudineus*, which is the test species in the present study, were also found. Akter et al. (2008) investigated acute toxicity of arsenic and mercury to the freshwater climbing perch, *Anabas*
testudineus (Bloch) and suggested that the 96 h LC$_{50}$ value obtained may be used as incipient LC$_{50}$ or lethal threshold concentration which can imply the toxicity strength of the pollutants and hence may be used as a measure of indication of pollution in the aquatic environment. Benli et al. (2009) studied the acute toxicity of deltamethrin on Nile Tilapia (Oreochromis niloticus L. 1758) larvae and fry and determined the 48 h LC$_{50}$ values of deltamethrin for the fish larvae and fry as 1.17 and 1.70 µg l$^{-1}$, respectively, thereby reporting that the pesticide was highly toxic to fish early life stages under acute exposure. Studies on the acute toxic effects of several other synthetic pyrethroids on different species of fish were also conducted. Clark et al. (2009) studied the toxicity of several pyrethroids (permethrin, fenvalerate, cypermethrin and flucythrinate) to marine invertebrates and fish and revealed that crustaceans were more sensitive than fish while oysters were comparatively less sensitive. Marigoudar et al. (2009) investigated mortality of Labeo rohita in different concentrations of cypermethrin at 96 h exposure period and reported that cypermethrin was very toxic even at lower concentration (4 µg l$^{-1}$) for 96 h LC$_{50}$ and further revealed that the lethality could be attributed to the inability of the fish to withstand and metabolize the cypermethrin intoxication. It further showed strong negative effects on survival as pesticide concentration increased. Davoodi and Abdi (2012) carried out a comparative study on the acute toxicity of synthetic pyrethroid pesticides permethrin 25%, monocrotophos 36%, and neem-based pesticide, neem gold E.C. 0.03%, to juvenile Cyprinus carpio Linn. and reported 96 h LC$_{50}$ values of 75.49, 72.26, and 56.89 mg l$^{-1}$ for neem gold, monocrotophos and permethrin, respectively, which concluded that the application of neem gold bio-pesticide for the control of unwanted organisms in agricultural farms is safer and more environmental friendly than synthetic pyrethroid pesticides. Khalili et al. (2012) studied the acute toxicity of diazinon and deltamethrin on swordtail fish (Xiphophorus helleri) where the 96 h LC$_{50}$ indicated that these
two pesticides were highly toxic to the fish. Tarkhani et al. (2012) also determined the acute toxicity of deltamethrin and diazinon on zebra fish, Danio rerio, where the LC$_{50}$ values obtained indicated that mortality rate of zebra fish exposed to both the pesticides increased with increasing concentration and time of exposure and further revealed that the fish was more sensitive to lower values of deltamethrin compared to those of diazinon. An examination of acute toxicity of deltamethrin in a riverine fish, Rhamdia quelen, native to Southern Brazil with emphasis on clinical, biochemical and haematological effects were also carried out which revealed that deltamethrin was highly toxic to this species. Because of its lipophilic nature it could be absorbed by the fish gills which partially explains the high sensitivity of these animals to deltamethrin exposure in concentrations up to a thousand times lower than in mammals (Galeb et al., 2013). The median lethal concentrations (LC$_{50}$) of deltamethrin and permethrin for Anabas testudineus were found to range between 0.07-0.11 mg l$^{-1}$ and 0.93-2.07 mg l$^{-1}$, respectively (Sapana Devi and Gupta, 2014).

2.3. Chronic Toxicity

2.3.1. Growth and food consumption

Sublethal concentrations of pollutants including pesticides have adverse effects on aquatic organisms in their growth and feeding rate. Therefore, several researchers have investigated the impact of toxicants including pyrethroid insecticides on growth and food consumption in aquatic organisms mainly fish. Grant and Mehrle (1973) reported inhibition in growth of fish, Salmo gairdneri at higher sublethal doses of endrin pesticide. In addition to this, Kumaraguru and Beamish (1986) also studied the effect of permethrin (NRDC-143) on growth of the same fish,
*Salmo gairdneri* where significant reduction in growth rate observed when exposed to sublethal concentrations of permethrin. Hay (1989) investigated the toxicity of pyrethroid insecticides to fish and reported that fish exposed to sublethal concentrations of pyrethroids resulted in decreased growth and impaired swimming performance in the fish. De Boeck *et al.* (1997) investigated the effects of sublethal copper exposure on accumulation, food consumption, growth, energy stores and nucleic acid content in common carp and revealed that higher concentrations of copper exposure affected both growth and feeding behaviour in common carp, whereas at lower concentration growth was affected despite normal food consumption and metabolic demand for the fish increased, challenging the carp with an increased demand for food. Aguigwo (2002) investigated the toxic effects of cymbush (cypermethrin) pesticide on growth and survival of the African cat fish, *Clarias gariepinus* (Burchell) which suggested that the growth parameters such as specific growth rate, food conversion efficiency and protein efficiency ratio decreased as the concentrations of cypermethrin increased. Studies on the effects of pesticides other than pyrethroids on aquatic organisms including fish were also found. Huang and Chen (2004) investigated the effect of sublethal concentrations of chlordane and lindane on growth rate in juvenile green-neon shrimp (*Neocaridina denticulata*) and reported that the growth rate of the exposed shrimp were lower than those of the control group. Yuan *et al.* (2004) compared the responses of a freshwater shrimp (*Macrobrachium nipponense*) including food consumption and body weight change after paraquat and malathion exposure and further reported that the effects of these two pesticides on the feeding rate were remarkable and body weight decreased when the concentration of the two pesticides increased in which paraquat had a stronger effect than malathion. Khan and Law (2005) examined the adverse effects of pesticides and related chemicals on enzyme and hormone systems of fish which further revealed that fish
exposed to environmental pollutants including pesticides were shown to reduce growth rate in the fish larvae. Hanson et al. (2007) studied the effects of some selected pesticides on the growth and reproduction of freshwater fishes *Oreochromis niloticus*, *Chrysichthys nigrigiradigitatus* and *Clarias gariepinus* and indicated that the pesticides had adverse effects on the general growth and reproduction of the species as shown by gonadosomatic indices. Yaji and Auta (2007) studied sub-lethal effects of monocrotophos on growth and food utilization of the African cat fish *Clarias gariepinus* (Teugels) and revealed that the growth rates were significantly reduced in fish exposed to the toxicant compared with the control groups. Studies on the effects of other environmental pollutants like herbicide and heavy metals on growth of fish were also found. Gad and Saad (2008) studied the effect of phenol on some physiological parameters of *Oreochromis niloticus* which revealed that different sublethal concentrations of phenol causes decrease in fish growth and it is directly proportional to phenol concentration and time of exposure. Bose et al. (2011) investigated the toxic impact of thiamethoxam on the growth performance of a freshwater fish *Oreochromis niloticus* which revealed that various sublethal doses of thiamethoxam had significant impact on growth of the fish where weight, length and breadth of the fish gradually decreased with increased dose of thiamethoxam. Richterová and Svobodová (2012) in a review on the influence of synthetic pyrethroids in fish stated that chronic effects of pyrethroids induced decreased growth in fish. Saha and Kaviraj (2013) examined dietary ascorbic acid as a means to counter the stress of pyrethroid cypermethrin on the growth of freshwater catfish *Heteropneustes fossilis*.

2.4. Biochemical Studies
Many studies have been found analyzing the effects of pesticides including synthetic pyrethroids on biochemical parameters like glycogen, total protein and lactic acid level in different tissues of several freshwater fish species including *Anabas testudineus*. Bakthavathsalam and Reddy (1982) investigated changes in the content of glycogen and lactic acid in liver and muscle tissues of *Anabas testudineus* (Bloch) exposed to furadan in which significant differences were observed in glycogen metabolism of the fish exposed to an acute lethal and a sublethal concentration of furadan. Bakthavathsalam and Reddy (1983) also examined the effects of lethal (0.59 mg l$^{-1}$) and sublethal (0.075 mg l$^{-1}$) concentrations of lindane in glycogen (liver and muscle), glucose (blood) and lactic acid levels (liver, muscle and blood) of the climbing perch, *Anabas testudineus*. Radhaiah and Jayantha Rao (1990) investigated toxicity of pyrethroid insecticide fenvalerate to glycogen content in muscle tissue of freshwater fish, *Tilapia mossambica* (Peters) where significant decrease in the level of glycogen was observed. Al-Akel *et al.* (1995) studied the effect of permethrin (NRDC-143) on the glycogen content in different tissues of freshwater fish *Oreochromis niloticus* where marked decline in glycogen content was observed in different fish tissues. Kumar *et al.* (1999) studied the impact of deltamethrin on physiological changes in freshwater catfish *Heteropneustes fossilis* in which depletion of lactic acid level in blood, liver, muscle, heart, gills, kidney and spleen was noticed after 30 days of deltamethrin exposure. Das and Mukherjee (2003) examined the effects of sublethal concentrations of cypermethrin on protein level in muscle and serum of the freshwater fish *Labeo rohita* in which significant reduction was found in serum protein. Svobodová *et al.* (2003) studied the effect of deltamethrin on haematological indices of Common carp (*Cyprinus carpio* L.) where plasma total protein was found to decrease as a result of deltamethrin exposure which may be referred to as possible disruption of proteosynthesis. Begum (2005) estimated in vivo biochemical changes in liver and
gill of *Clarias batrachus* during cypermethrin exposure and following cessation of exposure in which protein content in liver tissues decreased at the end of 1st and 5th day followed by slight increase at the end of 10 days while gill tissue showed statistically significant decrease in protein content during exposure period of 10 days. However, recovery in protein content was observed to a great extent in both the tissues. Begum (2007) investigated cypermethrin-induced biochemical perturbations in freshwater fish *Clarias batrachus* at sublethal exposure. Reduction in protein was observed in muscle and kidney tissue exposed to cypermethrin for 10 days in which recovery response was also seen in the tissues. Borges et al. (2007) studied changes in hematological and serum biochemical values in a riverine fish, *Rhamdia quelen* due to sub-lethal toxicity of cypermethrin in which reduction in total protein in serum was observed. Korkmaz et al. (2009) studied cypermethrin-induced biochemical changes in Nile tilapia (*Oreochromis niloticus*), and the protective and recuperative effect of ascorbic acid in which highest depletion of protein level in liver, muscle and gill tissues were found in 0.44 µg l⁻¹ cypermethrin + ascorbic acid supplemented diet group, followed by 0.22 µg l⁻¹ cypermethrin + control diet group, and 0.44 µg l⁻¹ cypermethrin + control diet group respectively after 10 days exposure. Singh et al. (2010) investigated toxicological and biochemical alterations of cypermethrin (synthetic pyrethroids) against freshwater teleost fish *Colisa fasciatus* in different seasons and reported that sublethal doses of cypermethrin after 96 h exposure significantly altered the level of total protein in muscle and liver tissues of the fish. Anita Susan et al. (2010b) examined the biochemical changes in the tissues of *Labeo rohita* and *Cirrhinus mrigala* exposed to fenvalerate where increase or decrease in the levels of glycogen in liver, muscle, gill, brain and kidney tissues in the two fishes were observed in response to lethal and sublethal concentrations of fenvalerate while decreased protein level was observed in most tissues of the two fish treated
with both lethal and sublethal concentrations of the pesticide. However, a slight increase in protein level was found in muscle and brain tissues of Cirrhinus mrigala treated with lethal dose of the pesticide. Firat et al. (2011) conducted a comparative study on the effects of a pyrethroid (cypermethrin) and two metals (copper, lead) on serum biochemistry of Nile tilapia, Oreochromis niloticus, where total protein level was found to decrease in cypermethrin and lead exposed fish after 21 days. Prusty et al. (2011) examined the effect of short term exposure of fenvalerate on biochemical and haematological responses in Labeo rohita fingerlings where serum total protein level was found to decrease significantly in response to fenvalerate exposure. Sharma and Ansari (2011) examined the effect of deltamethrin and a neem based pesticide ‘Achook’ on some biochemical parameters in liver, ovary and muscle tissues of zebrafish Danio rerio (Cyprinidae) where the protein content in liver, ovary and muscle tissues was reduced to 45, 68, and 65% after deltamethrin exposure and 54, 81, and 85%, respectively, after achook treatment for 16 days. Tantarpale (2011) investigated the impact of cypermethrin on total protein content in muscle and liver tissues of the freshwater fish Channa striatus in which the reduction of total protein was found more in muscle than in liver tissues at different exposure period of the pesticide. Vani et al. (2011) studied deltamethrin induced alterations in haematological and biochemical parameters in fingerlings of Catla catla and their amelioration by dietary supplement of vitamin C where the findings showed that deltamethrin had negative effect on haematological and biochemical parameters of the fish. Ahmad et al. (2012) investigated the effect of the pyrethroid lambda-cyhalothrin on some biochemical parameters in the gill, liver and ovary of zebrafish, Danio rerio (Cyprinidae) where the total protein content was reduced to 38, 46 and 45% in gill, liver and ovary, respectively, after lambda-cyhalothrin exposure for 21 days at the LC40 dose. Mohapatra et al. (2012) studied fenvalerate induced stress mitigation by dietary
supplementation of multispecies probiotic mixture in a tropical freshwater fish, *Labeo rohita* (Hamilton) in which total protein value was found to be reduced significantly in the fenvalerate exposed fish as compared to the probiotic supplemented fish. Suneetha (2012) studied the effects of the synthetic pyrethroid fenvalerate on carbohydrate metabolism of the freshwater fish, *Labeo rohita* (Hamilton) which resulted in decreased glycogen levels in brain, gill, kidney, liver and muscle tissues on exposure to the pesticide. Vani et al. (2012) studied alterations in haematological and biochemical parameters of *Catla catla* exposed to sublethal concentration of cypermethrin in which total serum protein was significantly reduced in cypermethrin exposed fish. Loteste et al. (2013) examined the sublethal effect of cypermethrin on protein level in liver tissue of the fish, *Prochilodus lineatus* (Valenciennes, 1836) which resulted in decreased protein content in the tissue of the exposed fish.

### 2.5. Enzyme Assays

Several enzyme studies in different fish tissues in response to synthetic pyrethroids were found. A review of the effects of pyrethroids including deltamethrin exposure in the respiratory enzymes succinate dehydrogenase (SDH), and two metabolic enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in several freshwater fish and invertebrate tissues is provided here.

#### 2.5.1. Succinate dehydrogenase (SDH)

Radhaiah and Jayantha Rao (1990) studied the toxicity of the pyrethroid insecticide fenvalerate in a freshwater fish, *Tilapia mossambica* in which significant decrease in the levels of succinate dehydrogenase was observed under fenvalerate intoxication. Singh and Agarwal (1993)
investigated the toxic effects of cypermethrin on lactate dehydrogenase, succinic dehydrogenase and cytochrome oxidase in the foot and hepatopancreas of snail, *Lymnaea acuminata*, Lamarck (Lymnaeidae) and the liver and muscle tissues of fish, *Channa striatus* (Bloch). Philip *et al.* (1995) studied cypermethrin-induced in vivo alterations in the carbohydrate metabolism of a freshwater fish, *Labeo rohita* in which the TCA cycle enzyme succinate dehydrogenase was found to be inhibited. Kamalaveni *et al.* (2001) examined the levels of succinate dehydrogenase (SDH) in various tissues of *Cyprinus carpio* exposed to lethal concentrations of pyrethroids (deltamethrin, cypermethrin, fenvalerate and fluvalinate) for a period of 72 h which resulted in a steady decrease in SDH activity indicating inhibition of SDH at mitochondrial level. Das and Mukherjee (2003) studied the sublethal toxic effects of cypermethrin on succinate dehydrogenase level in brain, liver and kidney tissues of *Labeo rohita* fingerlings which resulted in depletion of the enzyme in the exposed fish. Singh and Singh (2004) examined the biochemical changes in liver, brain, muscle and gill of *Catla catla* exposed to sublethal concentrations of alphamethrin in which SDH enzyme activity was found to decrease with increased exposure period and maximum inhibition was observed in gill and muscle tissues. Singh *et al.* (2010) found that cypermethrin exposure of the freshwater teleost fish *Colisa fasciatus* in different seasons significantly altered the levels of SDH in nervous tissue of the fish depending on time and dose of the pesticide. Suneetha (2012) investigated the effects of endosulfan and fenvalerate on enzymes like SDH, MDH and LDH activities in freshwater fish, *Labeo rohita* (Hamilton) and revealed that both endosulfan and fenvalerate caused alterations in the carbohydrate metabolism of the fish, but comparatively fenvalerate treated fish tissues showed more inhibition in the activities of carbohydrate metabolic enzymes than that of endosulfan treated fish.
2.5.2. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT)

Bálint et al. (1995) studied the biochemical and subcellular changes in carp exposed to the organophosphorous methidathion and the pyrethroid deltamethrin where serum AST was found to increase in deltamethrin treated fish. Begum (2005, 2007) studied the effect of cypermethrin on biochemical parameters like AST and ALT enzyme activities in liver, gill, muscle and kidney tissues of the fish _Clarias batrachus_ exposed to sublethal concentrations of the pesticide for 10 days in which increased enzyme activities were observed in all the tissues examined. Borges et al. (2007) investigated the sublethal effect of cypermethrin on serum biochemical parameters in fish _Rhamdia quelen_ in which significant increase in AST and decrease in ALT levels were recorded. Moreover, significant increase in serum AST and ALT activities were observed in Nile tilapia, _Oreochromis niloticus_ on exposure to deltamethrin (El-Sayed et al., 2007). Veříšek et al. (2007) studied the effects of deltamethrin on rainbow trout (_Oncorhynchus mykiss_) in which significantly lower values of plasma ALT and higher values of plasma AST were observed when treated with 96 h LC₅₀ value of the pesticide for 96 h. Firda et al. (2011) conducted a comparative study on the effects of a pesticide (cypermethrin) and two metals (copper, lead) on serum biochemistry including AST and ALT enzyme activities in Nile tilapia, _Oreochromis niloticus_, which showed that alterations in AST and ALT activities in pesticide-treated fish were higher than those in fish exposed to metals. Gabriel et al. (2011) studied the impact of cypermethrin on selected enzymes like AST and ALT in gill, liver, muscle and kidney tissues of _Heterobranchus bidorsalis_ and reported the usefulness of these two enzymes as biomarkers of cypermethrin toxicity which appeared to be concentration and tissue dependent, and could be effectively used to assess the impact of the agrochemical on the fish. Kumar et al. (2011) studied the alteration in nitrogen metabolism in freshwater fishes _Channa punctatus_ and _Clarias batrachus_ on exposure
to cypermethrin in which remarkable increase in AST and ALT were observed in brain, gill, liver, kidney and muscle tissues in response to cypermethrin exposure. Significant increase in serum AST and ALT level was also noticed in *Labeo rohita* fingerlings after 15 days exposure to fenvalerate indicating that fenvalerate can induce biochemical alterations in the fish in response to pesticide exposure (Prusty *et al*., 2011). Vani *et al*. (2011) examined deltamethrin induced alterations of hematological and biochemical parameters including AST and ALT enzyme activities in *Catla catla* fingerlings and their amelioration using vitamin C as dietary supplement. Yaji *et al*. (2011) studied the effects of cypermethrin on behaviour and biochemical indices of the freshwater fish *Oreochromis niloticus* in which serum total protein, AST and ALT levels decreased significantly with increase in concentration of the pesticide. Amin and Hashem (2012) studied deltamethrin-induced oxidative stress and biochemical changes in tissues and blood of catfish (*Clarias gariepinus*) in which significant increase in serum AST and ALT were observed. Gabriel *et al*. (2012) examined the changes in metabolic enzyme (AST, ALT) activities in gill, kidney, liver, muscle and plasma of *Clarias gariepinus* exposed to cypermethrin which revealed that the activities of the enzymes in all the organs showed concentration dependent activities which decreased significantly with increasing concentration of cypermethrin. Vani *et al*. (2012) investigated the effect of sublethal concentration of cypermethrin on biochemical parameters of the Indian major carp, *Catla catla* fingerlings in which a marked increase in AST and ALT activities in liver was observed. Galeb *et al*. (2013) examined the acute effects of deltamethrin in some hepatic enzymes such as AST and ALT in a riverine fish, *Rhamdia quelen* where significant decrease in the two enzyme activities was observed due to deltamethrin exposure. Loteste *et al*. (2013) also studied hepatic enzyme activity in the fish *Prochilodus lineatus* after sublethal exposure to cypermethrin where the results revealed significantly higher level of AST
and ALT in hepatic tissue at different concentrations of cypermethrin. Muthuviveganandavel et al. (2013) investigated the effect of synthetic pyrethroids on blood plasma biomarker enzymes and histological changes in *Catla catla*.

Although there are a number of studies on the effects of pyrethroids on key respiratory enzyme SDH and other metabolic enzymes like AST and ALT activities in different fish species, studies of the effects of deltamethrin and permethrin on the above mentioned enzymes in liver, muscle, brain, gill, heart and kidney tissues of freshwater fish are scant.

**2.6. Oxygen Consumption**

Studies on oxygen consumption uptake in aquatic organisms especially fish under stress is considered to be one of the reliable toxicological end-points. Several studies have been conducted to analyze the effects of toxicants including pesticides on oxygen uptake in fish. Bradbury et al. (1986) also studied the evaluation of gill uptake and toxicokinetics of fenvalerate in rainbow trout (*Salmo gairdneri*). Radhaiah and Jayantha Rao (1990) suggested that pyrethroid insecticide fenvalerate exposure caused decreased oxygen consumption in *Tilapia mossambica* with an increase in fenvalerate concentration. Besides pyrethroids, other pesticides and heavy metals also caused reduction in oxygen consumption of different fish species. Chinni et al. (2000) examined oxygen consumption in *Penaeus indicus* postlarvae exposed to lead in which the results revealed that a minimum decrease in oxygen uptake was observed after 24 h lead exposure and a maximum decrease after 30 days. Effect of fenvalerate on oxygen consumption had also been studied by other workers in the fish *Channa punctatus* (Bloch) and *Cirrhinus mrigala* (Tilak and Satyavardhan, 2002; Mushigeri and David, 2003). Some investigations on
effects of pesticides chlordane, lindane, paraquat and malathion on oxygen consumption in freshwater shrimp *Macrobrachium nipponense* and *Neocaridina denticulata* were also carried out where the results demonstrate that the pesticides had adverse effects in the normal oxygen uptake in the shrimps examined (Huang and Chen, 2004; Yuan et al., 2004). Joshi and Kulkarni (2007) examined the changes in the oxygen consumption of a freshwater fish *Garra mullya* (Sykes) exposed to cypermethrin and fenvalerate where the results indicated that the rate of oxygen consumption increased in the initial period of analysis in lethal and sublethal concentrations of both the pesticides and thereafter started decreasing, implying that the two pesticides caused decrease in oxygen uptake efficiency of the fish. Tilak and Swarna Kumari (2009) investigated the acute toxicity of Nuvan, an organophosphate in freshwater fish *Ctenopharyngodon idella* and its effects on oxygen consumption. They observed reduction in oxygen consumption thereby causing the death of the fish. Chebbi and David (2010) studied the alterations in the levels of ions and whole animal oxygen consumption of freshwater fish, *Cyprinus carpio* on exposure to quinalphos pesticide. Anita Susan et al. (2010a) conducted oxygen consumption studies for a period of 12 h, at intervals of 2 h in both sublethal and lethal concentrations of fenvalerate in the three major carps *Labeo rohita* (Ham.), *Catla catla* (Ham.) and *Cirrhinus mrigala* (Ham.). They observed decrease in oxygen uptake efficiency in all the three carps studied and further indicated that lethal concentrations had profound effects than sublethal concentrations; again 20% E.C. formulation of fenvalerate was found to be more deleterious than fenvalerate technical grade. MariyaDasu et al. (2013) investigated thiodicarb (larvin 75% WP) toxicity and its impact on biochemical changes and oxygen consumption of freshwater fish *Labeo rohita* (Hamilton). Hence, from the above studies, it is clear that pollutants including pyrethroids can impair oxygen uptake efficiency which may eventually be fatal to fish.
2.7. Scanning Electron Microscopic Studies of Fish Gill and Scale Tissues

Several studies have been found that reveal many specific effects of certain toxicants including pyrethroids on ultrastructures of different fish tissues such as gill and scale using scanning electron microscopy.

2.7.1. Gill tissue

Daoust et al. (1984) investigated the acute pathological effects of inorganic mercury and copper in gills of rainbow trout, *Salmo gairdneri*, inducing lesions that were most severe during the first 48 h of exposure to the metals, and were characterized primarily by apoptosis of lamellar epithelial cells and lamellar fusion as examined by light and electron microscopy. Kirk and Lewis (1993) studied the effects of pollutants (phenol, copper and ammonia) on the surface ultrastructure of the gills of rainbow trout using scanning electron microscopy, which revealed that although the gills exhibited some common pollutant-induced changes, certain type of pathological responses were pollutant-specific. Alazemi et al. (1996) examined gill damage in the freshwater fish *Gnathonemus petersii* (Family: Mormyridae) exposed to selected pollutants (cadmium, copper, chromium, cyanide and atrazine) using SEM in which specific alterations in gill ultrastructure were found to be associated with each toxicant. Dutta et al. (1996) studied the ultrastructural changes in the respiratory lamellae of the catfish, *Heteropneustes fossilis* after sublethal exposure to malathion. Dutta et al. (1997) examined the effects of diazinon on bluegill sunfish, *Lepomis macrochirus* gills using scanning electron microscopy where changes in the surface ultrastructure of the gill of the fish were observed following exposure to diazinon. Pfeiffer et al. (1997) studied the electron microscopic perspectives of gill pathology induced by 1-naphthyl-N-methylcarbamate in the goldfish (*Carassius auratus* Linnaeus). Çalta (1999)
examined the effects of toxic aluminium and low pH on gill development of rainbow trout (*Oncorhynchus mykiss*) larvae using scanning electron microscopy in which shortened and thickened filaments in the apical part and fusion of primary and secondary filaments and lamellae were observed. Khangarot (2003) also studied the mercury-induced morphological changes in the respiratory surface of an Asian freshwater catfish, *Saccobranchus fossilis* using scanning electron microscopy, which revealed that fish gill is morphologically and physiologically affected by a variety of mercury compounds present in the freshwater bodies. Machado and Fanta (2003) also investigated the effects of the organophosphorous methyl parathion on the branchial epithelium of a freshwater fish *Metynnis roosevelti*. Rao *et al.* (2003) studied the toxic effects of profenofos on tissue acetylcholinesterase and gill morphology in a euryhaline fish *Oreochromis mossambicus* and reported abnormal gill morphology with distinct breakages in gill arches and rakers along with deep lesions and erosions in the epithelium. David and Fontanetti (2005) used scanning electron microscopy to investigate the morphology of *Mytella falcata* gill filaments and to compare the gill structure in fish specimens from three sites of the Santos estuary in southeastern Brazil. Cengiz and Unlu (2006) also conducted a microscopic study on the sublethal effects of commercial deltamethrin on the structure of gill, liver and gut tissues of mosquitofish, *Gambusia affinis*. Johal *et al.* (2007) described the deleterious changes at ultrastructural level of the epithelial cells of the gill of *Cyprinus carpio communis* Linn. upon exposure to monocrotophos, resulting in different types of degenerations at a finer scale in gill. These in turn affected fish health drastically and altered the fitness of the fish in water even in the presence of insignificant amounts of this toxicant in the ambient water. Velmurugan *et al.* (2007a) studied the histopathological changes of fenvalerate on the gill, kidney, liver and intestine tissues of freshwater fish *Cirrhinus mrigala* by light microscopy.
Velmurugan et al. (2007b) also studied the histopathology of lambda-cyhalothrin on tissues (gill, kidney, liver and intestine) of Cirrhinus mrigala using light microscopy. Dey et al. (2009) reported some ultrastructural abnormalities of gill in Epizootic Ulcerative Syndrome affected Puntius ticto (Hamilton, 1822) which caused several effects on gill morphology like fusion of gill lamella, distortion of lamellar surface, damage of primary and secondary gill lamella and breakage and necrosis of gill racker. A number of surface microstructural abnormalities in the gill of a hill stream fish, Brachydanio rerio, inhabiting some streams of Meghalaya (a North East Indian State) were observed such as loss of alignment of primary and secondary gill lamella, deposition of mucous on lamella, fusion of gill lamella at places etc. (Prasad et al., 2011). Rajguru et al. (2011) reported the adverse effects in the ultrastructural features of gill in the freshwater fish, Anabas testudineus inhabiting a water body contaminated by bleached sulphite paper mill (Nogaon Paper Mill, Assam, India) effluents which revealed that the primary and secondary gill lamella of the fish had epithelial detachment at places in response to the pollutants. Yasser and Naser (2011) conducted a histopathological study on the impact of pollutants on fish collected from different parts of Shatt Al-Arab River and reported separation of lamellar epithelium, atrophy of secondary gill lamellae, necrosis of lamellar capillaries, loss of secondary lamellae and curving of primary lamellae in the gills of Cyprinus carpio due to certain water pollutants. Al-Ghanbousi et al. (2012) studied the histopathological changes in the structure of the gills of the fish, Aphanius dispar exposed to deltamethrin using light, transmission and scanning electron microscopy. Moitra et al. (2012) reported that the stress caused by endosulfan exposure induced damage in gill surface such as hemorrhage in primary lamella, epithelial necrosis, hypertrophy of the epithelial cells, rupture of gill epithelium, and sloughing of respiratory epithelium.
2.7.2. Scale tissue

Lepidological changes in *Channa punctatus* scale exposed chronically to sublethal levels of organochlorine pesticide endosulfan were studied and alteration in circuli shape and pattern in elemental deposition of scales was observed (Johal and Dua, 1994). Yoshitomi *et al.* (1998) studied the cadmium-induced scale deformation in carp (*Cyprinus carpio*) and reported that cadmium contamination had a morphological effect in the form of scale deformities. Dua and Gupta (2005) studied fish scales to assess mercury toxicology and revealed that mercury had a direct effect on fish scales and moreover, fish being a popular food for humans, mercury contaminated fish could prove dangerous for people’s health. Khanna *et al.* (2007) studied fish scales as bio-indicators of water quality of river Ganga and reported that the frequency and severity of damage to lepidonts was negligible or nil in the scales of fishes in less polluted or undisturbed areas whereas in extensively exploited areas by human activities, lepidonts were found to be either broken or damaged. Esmaeili and Gholami (2011) conducted scanning electron microscopy studies of scale morphology in the Cyprinid fish, *Rutilus frisii kutum* Kamenskii (Actinopterygii: Cyprinidae) as a means to assess the habitat condition of the carp particularly in habitats affected by pollution. Brarich and Jangu (2012) also studied the scales of *Cyprinus carpio* as heavy metal pollution indicator in the Harike wetland (Ramsar site). Çoban *et al.* (2013) examined the effects of chromium on scale morphology in scaly carp (*Cyprinus carpio* L.) using fish scale as a bio-indicator for heavy metal pollution which gradually increased in natural waters. Pala *et al.* (2013) carried out scanning electron microscopy studies of scales of a freshwater fish, *Channa gachua* inhabiting a north-east Indian hill stream contaminated by municipal wastes and other pollutants.
In the context of all the above mentioned studies documenting several deleterious effects in aquatic organisms including fishes on exposure to several pollutants particularly synthetic pyrethroid pesticides, the present study was conducted to analyze the effects of the synthetic pyrethroids deltamethrin and permethrin on different physiological parameters of *Anabas testudineus* in Cachar district, Assam, India, with an attempt to assess the risk of deltamethrin and permethrin exposure to fish in general and *Anabas testudineus* in particular.